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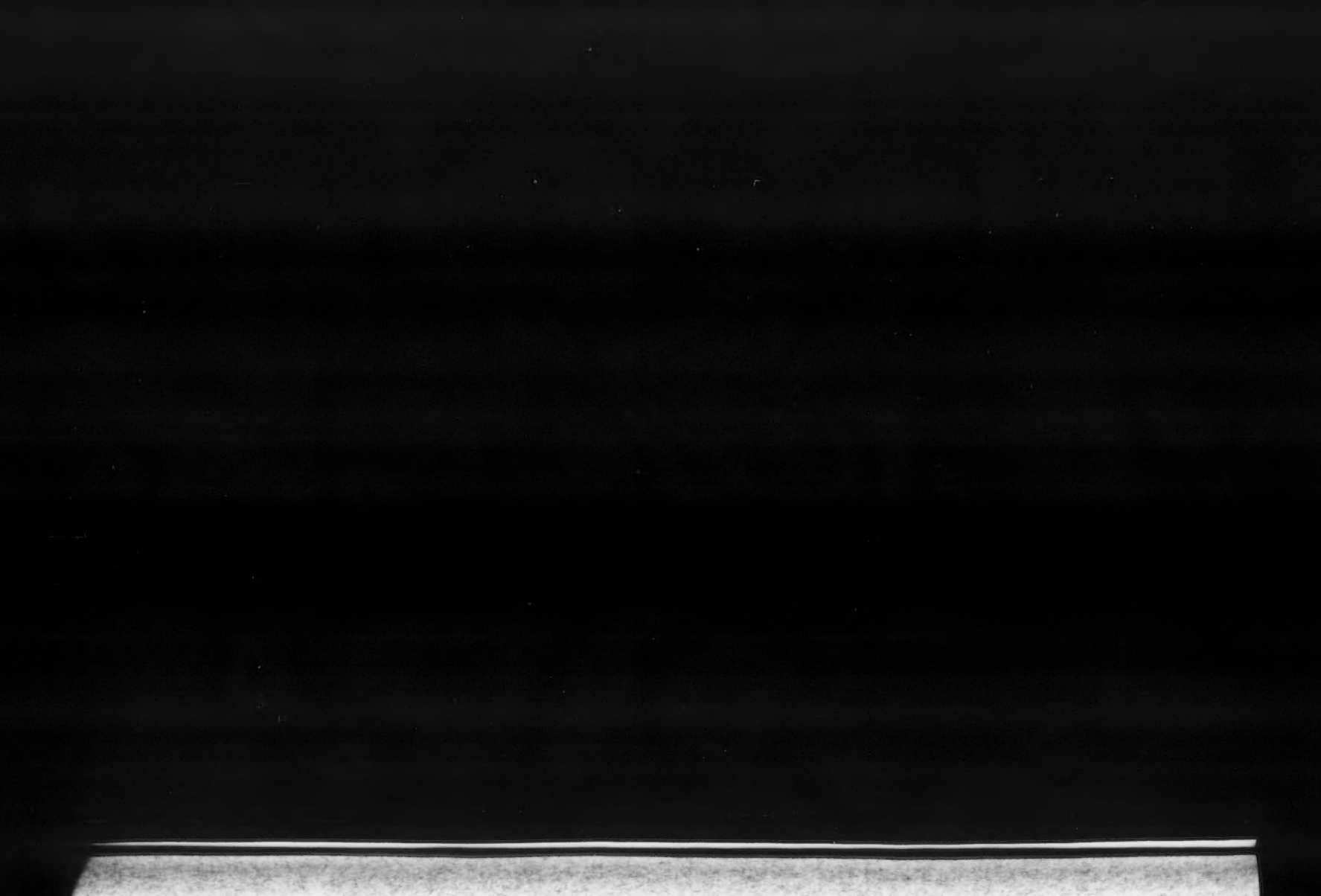

Vol. XLIII. *September, 1922.* *No. 3.*

BIOLOGICAL BULLETIN

SELECTIVE FERTILIZATION AND THE RATE OF POLLEN-TUBE GROWTH.

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A difference exists in the ability of pollen from dissimilar plants to accomplish sexual fusion when acting in competition. This has been shown by the writer (1920) with maize (*Zea mays* Linn.) and the tomato (*Lycopersicum esculentum* Mill.). Two other cases of a similar effect have been reported. These will be referred to later. In these species the plant's own pollen is more efficient in completing fertilization than pollen from plants having somewhat different genetical construction, and with maize the superiority of self-fertilization is greater as the germinal differences increase.

The method used to prove that there was an inequality in fertilization with maize was to make a mixture of approximately equal quantities of pollen from two different lots of plants, each of which possessed a dominant seed character. The mixture was applied at the same time to the plants which furnished the pollen, and when the seeds were mature it was possible to separate the seeds resulting from the two kinds of pollen on both types of plants. For example in some of the tests a white, smooth-seeded variety was contrasted with a yellow wrinkled variety. In one case the self-fertilized seeds were white and the cross-fertilized yellow, and in the other the contrast was between wrinkled and smooth seeds. The four classes of seeds in the two types of inflorescence were quite distinct and easily separated and counted. The numbers when arranged in the form of a proportion showed that there was a marked selective action in favor of self-fertilization.

The greatest difference in fertilizing efficiency was found when a small seeded variety of popcorn of the *Zea mays everta* type having very corneous and pointed seeds was used. The selective action was so pronounced that it seemed worth while to repeat the tests with this type on a more extensive scale. At the same time, the pollinations were made and the data recorded in such a way as to give an indication as to whether or not the result was due to a differential rate of pollen tube growth.

The seeds of maize are arranged regularly on a central spike. Each ovule has a separate pistil and these form a mass of fine filaments which extend beyond the enclosing leaf sheaths. See Fig. 1. Just before the pollinations were made the pistils were cut off evenly at a short distance beyond the tip of the spike, and the pollen mixture was applied to the cut ends of the filaments. The distance that the pollen tubes had to travel to reach the ovules differed considerably in the case of the seeds produced in the tip of the spike as compared with those at the base. The mature pistillate inflorescences of the material worked with ranged from ten to twenty centimeters in length. At the time fertilization took place they were considerably shorter than this. It is estimated that the pollen tubes travelled through a distance which varied from about five to fifteen centimeters. If the plant's own pollen tubes grow faster than the foreign tubes, we would expect fewer cross-fertilized seeds at the base of the spike than at the tip.

Five different mixtures of pollen were made and applied to about ten plants of each of the two contrasted types. For the white, smooth-seeded type a first generation hybrid of two inbred strains of a variety of sharply pointed popcorn known as Squirrel Tooth was used, and for the yellow, wrinkled type another first generation hybrid of two inbred strains from a variety of sweet-corn known as Golden Bantam. Hybrid plants were employed because of their size and vigor, making it possible to secure a large number of seeds from a single application of pollen. The plants of each type were characteristically uniform, and were practically identical in genetical constitution. They were producing segregating gametes but these were presumably alike in respect to their cytoplasmic covering. In any case the gametic differences were no greater than are present in the original varieties. The

same two types of plants were used in all five pollen mixtures. The mature inflorescences resulting from the application of mixed

FIG. 1. The pistillate inflorescence of maize at the time of pollination with leaf sheaths removed. (About one half natural size.)

pollen were divided into two approximately equal halves and the

seeds from the top half and bottom half were counted separately. The data from the entire spikes are brought together and given in Table I. to present the amount of selective action shown. The

TABLE I.

THE AMOUNT OF SELECTIVE ACTION SHOWN BY MAIZE IN FIVE POLLEN MIXTURES.

Pollen Mixture No.	Number of Seeds.				Total Number of Seeds.	Deviation from Perfect Proportion in Per Cent.
	A × A.	A × B.	B × A.	B × B.		
1	811	11	381	2,006	3,209	41.35
2	4,222	27	466	1,404	6,119	37.22
3	1,568	2	319	224	2,113	20.56
4	1,930	29	73	309	2,341	39.71
5	4,084	6	963	290	5,343	11.50

figures from all the plants used with each mixture are combined and are arranged in four classes in the form of a proportion. If there were no differences in fertilizing ability the proportion should be a perfect one within the limits of random sampling. The white, smooth-seeded plants are designated *A* in the tables, and the plants with yellow wrinkled seeds when uncrossed are listed under the heading of *B*. The number of seeds resulting from *A* pollen and from *B* pollen on *A* plants should be in the same ratio as the numbers from the same two kinds of pollen on *B* plants irrespective of the amount or viability of each kind of pollen in the mixture. As in the numerous cases previously reported such is clearly not the result obtained, as there are much fewer cross-fertilized seeds than would be expected if fertilization took place at random. The selective action is in favor of the homogeneous union as was found before and the differences are marked. The last column gives the deviation from the closest calculated perfect proportion based upon the per cent. of cross-fertilized and self-fertilized seeds on each type of plant. The maximum deviation possible is 50 which would indicate complete functioning of each kind of pollen on its own flowers, but not at all on the others, or vice versa as the case might be. The deviations actually range from 12 to 41 in favor of self-fertilization, in each case, and show a very high degree of selective action, amounting in some cases to almost total inability of the extrane-

ous pollen to bring about fertilization when acting in competition. Fig. 2 shows a pair of mature pistillate inflorescences resulting

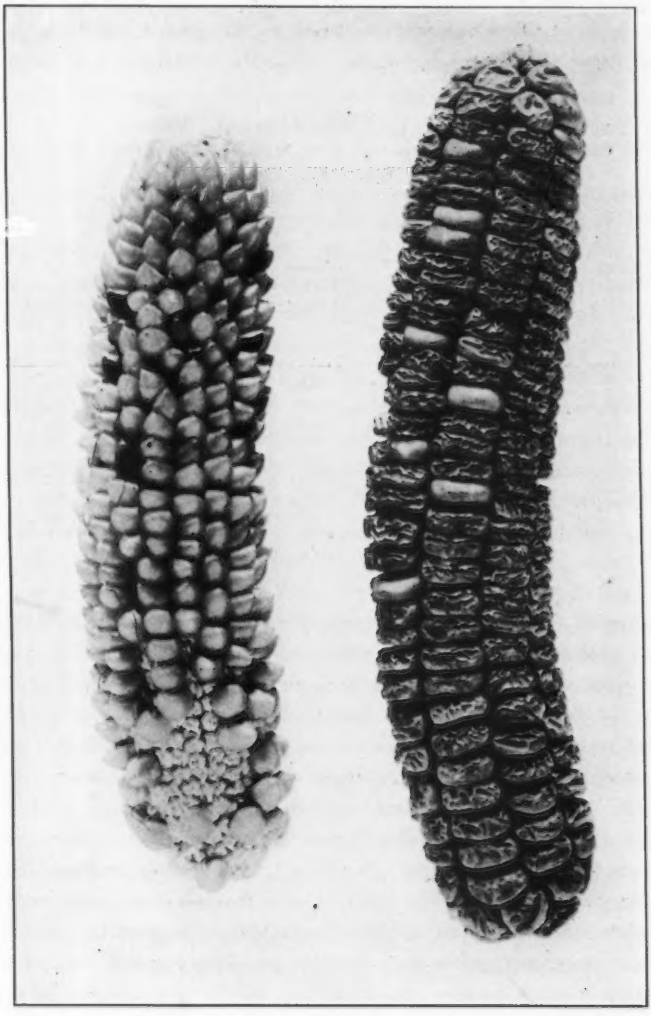

FIG. 2. Mature inflorescences resulting from the application of a mixture of pollen from the two kinds of plants which produced them. (About three fourths natural size taken on orthochromatic plate with Wratten C blue color filter.)

from pollen mixture number 1. The cross-fertilized seeds stand out unmistakably on both plants, but are few in number.

The data which show that this selective action is due in part, at least, to differences in rate of pollen-tube growth, are arranged in Table II. The total number of seeds in the top and bottom

TABLE II.

THE DISTRIBUTION OF CROSSED SEEDS IN MAIZE INFLORESCENCES RESULTING FROM MIXED POLLINATIONS.

Pollen Mixture No.	Plant Type.	Number of Seeds.		Total Number of Seeds.	No. of Crossed Seeds per Hundred.		Excess of Crossed Seeds per Hundred in Top Half.
		Top Half.	Bottom Half.		Top Half.	Bottom Half.	
1.....	*A*	452	370	822	1.99	.54	1.45
1.....	*B*	1,219	1,168	2,387	17.23	14.64	2.59
2.....	*A*	2,126	2,123	4,249	1.22	.05	1.17
2.....	*B*	979	891	1,870	26.66	23.01	3.65
3.....	*A*	811	759	1,570	.25	.00	.25
3.....	*B*	298	245	543	60.07	57.14	2.93
4.....	*A*	1,023	936	1,959	2.54	.32	2.22
4.....	*B*	186	196	382	24.73	13.78	10.95
5.....	*A*	2,075	2,015	4,090	.10	.20	−.10
5.....	*B*	612	641	1,253	78.43	75.35	3.08

halves of the inflorescences, as divided arbitrarily before shelling, are roughly equal. The number of cross-fertilized seeds per hundred of all seeds is more in those seeds which resulted from the shorter lengths of pollen tubes. Only one exception is noted, and here there were only six crossed seeds in a total of over four thousand. In all the others, positive differences are shown, but these, however, are not large, so that one cannot be sure whether or not the inequality in fertilization is due entirely to differences in the rate of pollen-tube growth. Corroborative evidence has been furnished by Miller (1919), who has observed that many pollen tubes may start to grow down the style of maize, but in about 100 examinations only one tube was seen to reach the ovary cavity in every case.

A similar selective action favoring the plant's own pollen has been found with cotton. Balls (1919) put an equal quantity of pollen of two distinct cultivated types of this plant (*Gossypium*)

on the stigmas of each. Only ten hybrids were obtained from 330 seeds resulting from the mixed pollen. Likewise Heribert-Nilsson (1920) found that the pollen tubes of *Œnothera gigas* grow slower in the styles of *O. Lamarckiana* than do the latter's own pollen tubes. These results were obtained by cutting off the styles close to the ovary at different times after pollination and noting the shortest time after pollination necessary for seeds to set.

A somewhat different kind of selective fertilization has been demonstrated by Correns (1920) with the dicecious plant *Melandrium*. The staminate plants of the species worked with are heterogamous, and the pistillate plants homogamous, corresponding to the sex conditions in animals of the *Drosophila* type. The pistillate-determining pollen tubes apparently grow faster than those which result in staminate plants. This was demonstrated in the following way. The mature seed-pods were divided into upper and lower halves and the seeds of each grown separately. The seeds from the upper portion resulting from the shorter lengths of pollen tubes gave 68 per cent. pistillate plants, while the seeds from the lower portion gave 56 per cent. of the same type. It should be noted that the conditions are reversed in the case of *Melandrium* and *Zea*. In the former the pollen tubes enter the ovary at a common point and are free to fertilize the first ovules they reach. The tubes which grow fastest therefore fertilize the ovules in the upper part of the ovary, leaving the slower-growing tubes to pass on down to the lower part. In *Zea* each ovule has a separate style so that, the longer the distance to traverse is, the less chance will the slower-growing tubes have of reaching the goal first.

Correns also found that when pollen was applied in large excess as compared to a moderate application, the pistillate plants resulting were always in greater proportion. But even when a deficient amount of pollen was applied there was still a small excess of pistillate plants showing that some other selective factor than differences in rate of pollen-tube growth was operating in addition.

Heribert-Nilsson also obtained aberrant ratios from self-fertilized and back-crossed heterozygous red-nerved plants of *Œno-*

thera Lamarckiana. He interprets the result as due to a selective action between gametes carrying different factors. In this case as well as in the preceding experiments with *Melandrium* two kinds of gametes were produced by the same individual and presumably they were alike in respect to their cytoplasmic covering, differing only within the nucleus. In the case of *Zea, Lycopersicum, Gossypium,* and *Œnothera Lamarckiana* pollinated by *gigas,* the gametes came from different individuals of unlike germinal construction and therefore differed both in nuclear content and enveloping cytoplasm. The two manifestations of selective fertilization may be unrelated. On the other hand it is not unlikely that they may have something in common.

The discrimination which works against the bringing together of individuals of unlike germplasm has been demonstrated by representatives of four distinct orders in the two main classes of angiosperms. In its effect it is comparable to the assortative mating of animals from the lowest to the highest. The predilection for the bringing together of like with like is more evident as the germinal differences increase. There is thus exhibited a tendency which when carried far enough may erect an impassable physiological barrier between different groups that were once compatible. It is an indication that sterility between species is the result of the accumulation of genetic differences, however these differences may arise.

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THE TEMPORARY CONCENTRATION OF SEA-SALTS ABOUT *ARBACIA* EGGS.

OTTO GLASER.[1]

I. Introduction.

If the precipitates prepared by Miss Woodward[2] and myself[3] have been correctly understood, Lillie's hypothetical fertilizin[4] is a mixture of at least two chemical entities. On this view, therefore, we cannot assume that the sperm-agglutination test, however reliable for agglutinin, is necessarily also a measure of the concentration of the associated lipolysin. The ratio $\frac{\text{agglutinin}}{\text{lipolysin}}$, may of course be a constant; yet we cannot know this until we find out.

With this problem in mind, I began, last summer, to search for other methods; the ideal being some convenient procedure which would eliminate the physiological variables and leave to the observer nothing except a reading. Experiments on specific gravity, surface tension and viscosity naturally suggested themselves. Changes in one or all of these properties of sea-water might be expected as exudate leaves the eggs and becomes distributed in the solvent. Such changes, plus or minus, should stand, within certain limits, in some direct or inverse relationship with the concentration of the organic constituents of the secretion. A comparison of such values with results gotten by the sperm-agglutination test for the same secretions and for solutions of precipitated agglutinin having the same specific gravity, surface tension, or viscosity, could then be used to throw light on the ratio $\frac{\text{agglutinin}}{\text{lipolysin}}$.

[1] From the Biological Laboratory of Amherst College, Amherst, Mass.

[2] Woodward, A. E., Studies on the Physiological Significance of Certain Precipitates from the Egg-Secretions of *Arbacia* and *Asterias*. J. Exp. Zoöl., Vol. 26, p. 459.

[3] Glaser, O., The Duality of Egg-Secretion. Am. Nat., Vol. LV., p. 368.

[4] Lillie, F. R., The Mechanism of Fertilization. Science, Vol. XXXVIII, p. 524.

II. The Diluent Effect of *Arbacia* Eggs.

I tried practically all the familiar variants of the drop and capillary methods for surface tension; the rates of flow, of falling plungers, and of capillary rise, for viscosity; the Westphal balance, for specific gravity. Yet despite the precautions taken to insure comparable measurements, my results remained inconsistent. Even the specific gravity readings were irregular and their sense totally contrary to expectations. In fact the specific gravity of sea-water in process of receiving exudate from the eggs, rarely rose, never remained constant, and almost invariably fell. The records in Table I. show the discrepancies.

Table I.

No.	Ratio.	Secretion Time.	Temperature.	Specific Gravity.	
				Sea-Water.	Exudate.
A.....	$\frac{\text{eggs}}{\text{sea-water}}$ = 1/10	1 hr.	23°	1.0226	1.0222
B.....	" = 1/10	1 hr.	23°	1.0226	1.0217
C.....	" = 1/10	1 hr.	23°	1.0226	1.0222
D.....	" = 1/10	2 hrs.	21.5°	1.0227	1.0231
E..	" = 1/10	30 min.	23°	1.0231	1.0222
E..	" = 1/10	1 hr.	23°	1.0231	1.0221
E..	" = 1/10	1½ hrs.	23.5°	1.0229	1.02195
E..	" = 1/10	2½ hrs.	23.7°	1.02275	1.0224

It is apparent that the introduction of *Arbacia* eggs into sea-water results in a slight decrease of specific gravity and that this decrease may be compensated or even over-compensated with the lapse of time. This is illustrated by series *E*, based on a single set of eggs, and by *D*. If their correctness could be established, these observations would account for the inconsistencies in surface tension and viscosity determinations; yet the fall in specific gravity, the irregularities in the magnitude of the fall, and the compensations would remain to be accounted for.

III. The Chlorine Deficit in Egg-Secretion.

There are four possible explanations. First, the specific gravity determinations may have been wrong. Secondly, the diluent effect of the eggs might be due to a liberation of heat. And finally,

the reductions in density may have resulted from one or the other or both of two causes; either salts are removed from the sea-water directly by the eggs, or the exudate itself affects the sea-water such that the specific gravity must fall.

The first two assumptions were entirely ruled out by subsequent developments. I shall limit myself therefore to a discussion of the other possibilities. Do *Arbacia* eggs abstract salts from the sea-water or is the reduction in specific gravity an effect traceable to the materials which the eggs secrete?

Just how the presence of exudate might reduce specific gravity, is more or less uncertain in detail. Nevertheless this possibility must be reckoned with, both in its thermal, as well as more narrowly chemical, aspects. Salts dissolved in water apparently bring about a "contraction" of the solvent.[5] Where ionization is incomplete, this effect, though marked, is not easily calculable; in dilute solutions, however, the total "contraction" is additively the sum of specific effects or moduli of the individual ions. Thus a gram-molecule of a salt with molecular weight M in m grams of water produces a change of volume Δ_v such that

$$\Delta_v = \frac{M - m}{S} - \frac{m}{S_o},$$

where S is the density of the solution at a given temperature and S_o the density of pure water at the same temperature.

Since undissociated molecules also have a "contractile" effect, and since each ion has a specific modulus, it would be quite possible to bring about a reduction in the specific gravity of sea-water by the addition of some agent that disturbs, selectively or otherwise, the ion-salt equilibrium. Our problem then narrows down to this: is the observed decrease in specific gravity associated with a genuine salt-deficit in the solution or is it the outcome, direct or indirect, of a physical-chemical rearrangement among the free solutes?

If real, and essentially non-selective, a salt-deficit in the solution should be detectable by the titration of the chlorides. For this purpose I used $n/20$ $AgNO_3$; and two or three drops of 10 per cent. K_2CrO_4, in doubly distilled water, as indicator.

[5] Nernst, W., Theoretical Chemistry. MacMillan and Co., London, 1895, p. 331.

Special precautions were taken since the specific gravity readings suggested that differences, if at all discoverable by titration, would be small. For this reason the measurements throughout were made with the same burettes and pipettes and comparable titrations always at the same temperature. Moreover it soon became apparent that the preparation of the eggs could not be carried out by any of the methods in ordinary practise. I therefore washed the sea-urchins first very thoroughly in a stream of running fresh water after which they were completely submerged in dishes for from three to five minutes. The bath was followed by partial drying and the complete removal of the spines by means of a coarse cloth. The naked tests were then carefully wiped with a clean towel and placed in an inverted position in individual Syracuse watch crystals. This procedure may appear cumbersome. However, it consumes very little more time than the usual methods of preparation and is the only way in which eggs absolutely free from detritus, traces of sea-water, dermal and other secretions, can be gotten. Incidentally the method has a further advantage; the brief immersion in fresh water causes the sea-urchins to shed their sexual products in unusual quantity and with the greatest promptness. Indeed one must work quickly in

TABLE II.

Series.	Ratio.	Secretion Time, Minutes.	Chlorine, in c.c. $n/20$ $AgNO_3$, per c.c.		Deficit per c.c. in 1/10 Milligrams of Chlorine.
			Sea-water.	Secretion.	
I.....	$\frac{\text{eggs}}{\text{sea-water}}$ = 1/7	15	10.5	10.3	−4
II.....	" = 1/7	15	10.5	10.4	−2
III.....	" = 1/30	60	11.0	10.9	−2
IV.....	" = 1/8	60	10.1	9.8	−5
IV.....	" = 1/8	60	10.0	9.8	−4
IV.....	" = 1/8	60	10.0	9.9	−2
IV.....	" = 1/8	60	10.2	9.8	−7
V.....	" = 1/3	60	10.5	10.1	−7
VI.....	" = 1/8	90	10.5	10.3	−4
VII.....	" = 1/20	120	11.0	10.9	−2
VIII.....	" = 1/8	150	9.9	9.8	−2
IX.....	" = 1/5	150	10.6	10.4	−4
IX.....	" = 1/5	150	10.6	10.5	−2
X.....	" = 1/8	180	10.5	10.4	−2
XI.....	" = 1/2	180	10.6	10.5	−2
XI.....	" = 1/2	180	10.7	10.4	−5

order to prevent the loss of good material through premature shedding of the eggs and sperm.[6]

With these precautions the titration of the chlorides yields perfectly consistent results.

Considering the sources of error and especially the difficulty of measuring the volume of a large number of eggs, the constancy in the sense of these differences is impressive. There is unquestionably a chlorine-deficit in egg-secretion.

IV. The Origin of the Chlorine-Deficit.

How does the chlorine deficiency arise? There is a presumption in favor of attributing it to the eggs; on the other hand, if these eliminate substances capable of masking the chlorine, $AgNO_3$ would give no more clue to its presence than in the titration of chloroform or trichloracetic acid. The problem is soluble by two very simple tests.

If the chlorine is removed by the eggs rather than masked by the exudates, it should be possible to prepare egg-secretions without a chlorine-deficit. To accomplish this the eggs should be exposed to sea-water until all the chlorine which they are able to hold has presumably been taken up. Such eggs if subsequently permitted to secrete into a fresh volume of sea-water should remove no chlorine whatever on their second exposure.

The reasoning is justified by the following experiment in which 1 c.c. of control sea-water, 1 c.c. of first sea-water and 1 c.c. of second, are all expressed in terms of $AgNO_3$ $n/20$.

Table III.

Ratio.	Time.	Control Sea-water.	1st Sea-water.	2d Sea-water.
$\frac{\text{Eggs}}{\text{Sea-water}} = 1/8$	11 A.M.	10.5	10.5	
	11:30	10.5	10.3 Eggs transferred to 2d Sea-water.	
	11:30			10.5
	12:00	10.5		10.5

[6] The efficacy of a three- to five-minute submersal in fresh water was first noticed by my colleague, Miss Sampson.

This result however does not yet solve the problem. It might be argued that the substance which masks the chlorine is secreted only by eggs newly shed from the ovary. If true, the failure to remove chlorine in the above experiment from the second volume of sea-water could be attributed to the absence of the chlorine-masking secretion.

A direct test of this idea is easily made, for if the deficit were, in reality, only apparent, it should be possible by complete evaporation to recover from equal volumes of sea-water and of secretion, equal quantities of sea-salts. This, as the following comparison shows, is not the case.

TABLE IV.

	Chlorine per c.c. as $AgNO_3$ $n/20$.	Total Salt per c.c.
Sea Water	10.6 c.c.	.0387 gram
Secretion	10.4 c.c.	.0370 gram

We must conclude then that the chlorine is not masked by the organic materials in the secretion, but is removed from solution by the eggs. Moreover, if we arbitrarily assume that the eggs remove only KCl, which in relation to its chlorine content is the heaviest of the salts present, there would still remain a discrepancy between the total salt-deficit of 1.7 milligrams per c.c. of secretion and the loss attributable to KCl alone. The total deficit therefore does not appear to result from a selective action on the part of the eggs. On the contrary, we must believe that all the salts are affected in proportion to their concentration in sea-water and their capacity for being removed by the particular egg-mechanism involved in the process.

V. THE MECHANISM BY WHICH SALTS ARE REMOVED FROM THE SEA-WATER.

Although an inspection of Table II. suggests that the chlorine deficit does not increase after the first fifteen minutes of exposure, the actual state of affairs can be rendered much clearer by reducing the values given to a common basis. We will assume an ample supply of chlorine; also, for the sake of comparison, that in one hour, 1 c.c. of eggs can remove from 1 c.c. of sea-water as much chlorine as from twenty; and further, that in 1 hour 1

c.c. of eggs removes one third as much as in three. On the basis of these assumptions and the actual titrations, we can construct a curve showing the comparative amounts of chlorine which, under the conditions imagined, 1 c.c. of eggs would abstract from 1 c.c. of sea-water in one hour, if the rate of removal for that hour were constantly the rate deducible from the determinations actually made after exposures of 15, 60, 90, 120, 150, and 180 minutes.

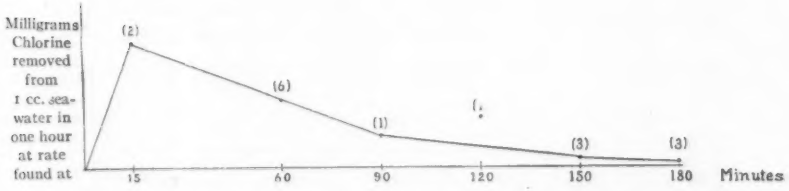

FIG. 1. Curve showing gradual loss in the power of eggs to abstract salts from sea-water.

The number of titrations upon which the points indicated in the curve rest, is given in brackets. Clearly there is a steady approach to zero. Since the rate of chlorine removal by eggs immersed for 180 minutes falls from the height which it had reached after 15 minutes it follows that the process of salt-abstraction is reversible. However, there is no equilibrium. We must suppose therefore that the chlorine-removing mechanism slowly breaks down.

Within the time-limit of these experiments, the only part of the egg-system known to undergo dissolution is the jelly which surrounds each newly shed egg. This chorion disintegrates slowly in sea-water. Fortunately it can also be readily removed by moderate shaking. I therefore compared the chlorine deficits produced by equal volumes of normal and dechorionized eggs in equal volumes of sea-water.

The experiment, of course, involves two errors: in the first place one cannot be certain that the jelly has actually been removed from every egg; and secondly, its removal from a considerable number renders it certain that a given volume of dechorionized ova will contain a larger number of cells than an

equal volume of normal eggs. Both errors really operate against any à priori idea that the jelly is responsible for the removal of the salts. If then the result is clear cut nevertheless, it would appear that the experiment is decisive.

Two such comparisons are given in Table V.

TABLE V.

CHLORINE PER C.C. IN TERMS OF $AgNO_3$ $n/20$.

	Sea-Water Control.	Sea-Water with Normal Eggs.			Sea-Water with Chorion-free Eggs.		
After 30 minutes	11.0	$\frac{\text{eggs}}{\text{sea-water}}$	$\frac{.2 \text{ c.c.}}{14.8 \text{ c.c.}}$	= 10.9	$\frac{\text{eggs}}{\text{sea-water}}$	$\frac{.2 \text{ c.c.}}{14.8 \text{ c.c.}}$	= 11.0
After 2 hours	11.0	$\frac{\text{eggs}}{\text{sea-water}}$	$\frac{.2 \text{ c.c.}}{10 \text{ c.c.}}$	= 10.9	$\frac{\text{eggs}}{\text{sea-water}}$	$\frac{.2 \text{ c.c.}}{10 \text{ c.c.}}$	= 11.0

A further test seemed desirable. If the jelly takes up the salts, it should be possible to demonstrate their presence in the chorion. The method was that of Macallum[7] in which the reagent, $n/10$ $AgNO_3$ is acidulated, per liter, with 25 c.c. of 60 per cent. HNO_3 in order to avoid any confusion that might result from possible phosphate precipitates or combinations of the silver with proteins or their constituent parts.

The eggs after being carefully drained were gradually transferred to absolute alcohol, and after hardening, subsequently treated with Macallum's reagent for half an hour. After this they were placed on a glass slide, cleared in glycerine under a cover slip, and exposed to direct sunlight for 30 minutes. The distribution of the reduced silver is shown in Fig. 2.

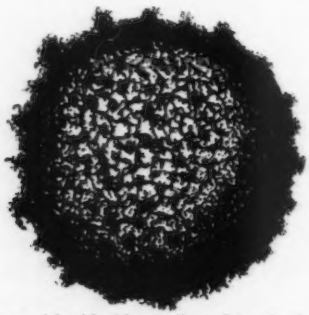

FIG. 2. *Arbacia* egg with chlorides indicated in chorion.

[7] Macallum, A. B., Die Methoden der Biologischen Mikrochemie. Abderhalden's Handb. d. Bio-chem. Arbeitsmethoden, 1912, p. 1100.

Conclusions.

Any attempt to standardize *Arbacia* egg-secretion by physical methods must take into account the fact that these eggs temporarily lower the specific gravity of sea-water. This renders unreliable any immediate application of methods depending on surface tension, viscosity, or specific gravity.

The decrease in density is accompanied by a measureable chlorine deficiency in the solution and by a greater shortage of total salt. We cannot attribute these effects directly or indirectly to the substances which the eggs eliminate since a preliminary exposure of the eggs to sea-water enables us to produce on second exposure a secretion without salt-deficit. Moreover, the results of total evaporation show that the salts were definitely out of the solution and that there is no selective abstraction by the eggs other than that dependent on the proportions in which the several salts are present and their capacity for being removed by the particular egg-mechanism which is involved in the process.

This mechanism is the chorion, for eggs deprived of their jelly by shaking do not cause a salt-deficit. Localization by means of $AgNO_3$ $n/10$ in the presence of HNO_3 demonstrates the concentration of chlorides about the eggs.

The concentration is temporary since the chorion within a few hours normally undergoes disintegration in sea-water. As the result of this the salts are dispersed and the specific gravity of the sea-water may return to normal. A rise above normal may be attributed to the presence of exudate in solution.

In time these facts may find an application in the theory of fertilization. For the present they are presented without theoretical bias although there are unavoidable suggestions in the fact that the concentration of sea-salts immediately about an *Arbacia* egg is temporarily and measurably greater than the concentration of the same salts in the surrounding sea-water.

March 1, 1922.

NOTES ON THE OCCURRENCE OF ABNORMAL MITOSES IN SPERMATOGENESIS.

ROBERT H. BOWEN.[1]

Mitotic figures abnormal particularly with respect to the number of chromosomes and centrioles involved are, as is well known, of common occurrence in the spermatogenesis of many insects. Apart from their interest as mere abnormalities, these cases sometimes throw light on more important problems of cell division and acquire, therefore, a much greater significance. In the course of several years' study of hemipteran (Family *Pentatomidæ*) spermatogenesis a variety of abnormal types of division have been observed, and it has seemed worth while to assemble descriptions of some of these, in particular those not hitherto described by other workers. The cases here reported are all from the male germ cells of various Hemiptera, belonging with one exception to the Family *Pentatomidæ*. The material was originally fixed for a variety of different purposes and the methods are accordingly noted separately where necessary. I am indebted to Professor E. B. Wilson for the use of a number of slides of *Loxa florida* which he had prepared some years ago and which I found presented a very unusual type of cell division.

Abnormal mitoses occur sometimes as solitary exceptions among many normal cells, but at other times they occur in large numbers in a single testis. In the latter case the abnormal cells may form a large percentage of the cells in a single spermatic cyst. Frequently the condition which produces these irregularities is more widely operative with the result that large numbers of cells in a given testicular lobe or follicle are involved. The isolated cases are doubtless due to occasional 'accidents' such as are to be expected in all vital phenomena; but the simultaneous occurrence of many abnormal divisions seems sometimes to depend on other causes, the nature of which is for the most part unknown.

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There are obviously three possible types of abnormal cell division, which may be outlined as follows:

Case 1.—The number of chromosomes is normal, but the number of centrioles is abnormal.

Case 2.—The number of centrioles is normal, but the number of chromosomes is abnormal.

Case 3.—The number of both chromosomes and centrioles is abnormal.

Examples of these three cases will be considered in order.

Case 1.

The occurrence of dividing cells in which a normal number of chromosomes is arranged on a multipolar spindle is very rare. Indeed, I have found only a single case of this kind, and do not recall having noted any similar cases in the published work of other writers. This unusual example was found in a testis of *Chelinidea vittiger* Uhl. (Family *Coreidæ*), which did not present any other unusual features. In the male of *Chelinidea* the spermatogonial chromosomes number twenty-one, of which two are very small—so-called *m* chromosomes—and one, an unpaired *X* chromosome. In the first maturation division the chromosomes, eleven in number, are arranged in the manner characteristic of other coreids.

The abnormal division here reported occurs in a cyst of spermatocytes in the metaphase of the first maturation division. The spindle is tri-polar (Fig. 3) and the centriole at each pole can be distinctly identified. The legs of the triangular spindle figure are nearly but not quite equal; otherwise it is very regular in formation. The eleven chromosomes characteristic of this division can be easily counted, and in most cases their distribution to the spindle poles can be foretold. Their arrangement on the spindle is very regular, and it will be readily seen that each pole of the spindle will receive approximately the same number of anaphase chromosomes. In the figure the chromosomes are numbered according to the approximate level of focus at which they appear, No. 1 being the topmost (the two topmost chromosomes (No. 1) were added from a contiguous section).

The disposition of the *m* chromosomes is particularly interest-

ing. Ordinarily they conjugate very briefly on the spindle (Wilson, '05*b*), and then separate precociously; but in this case they seem to have gone on the spindle entirely separate from each other (even their customary semblance of synapsis has failed). One gets the impression that they receive spindle fiber attachments at both ends, although a single one might be expected if such attachments are invariably specific.

The origin of the third centriole in a case of this kind is obscure, but it is possible that a tripolar spindle might arise through the precocious separation of one of the pairs into which the centrioles of the hemiptera are characteristically divided at an early stage in the growth period of the primary spermatocytes; but the staining of the centrioles in the case which I have described was not sufficiently precise to permit analysis of this possibility.

Case 2.

Cases in which normal, bipolar spindles are developed in connection with an unusual number of chromosomes are of not infrequent occurrence. The best known examples are to be found in triploid and tetraploid "mutants," in which the abnormal chromosome number exists in all the cells of the body. Similar multiplications of the normal chromosomal complex likewise occur in the germ cells of normal individuals with some frequency, either as isolated cases or in groups. Henking ('91), who first made a critical examination of hemipteran chromosomes (in *Pyrrhocoris*), seems to have noted a case of the occurrence of the diploid chromosome number in the first maturation division (Henking ('91), Fig. 30*a*), though his explanation of the make-up of the doubled chromosomes now seems doubtful. He noted also the occurrence of a tetraploid connective tissue cell, an abnormal condition commonly (if not constantly) present in the cells which form the sheath of the hemipteran testis and its smaller subdivisions. Hartman ('13) has more recently reported several cases in the spermatogonia and primary spermatocytes of the grasshopper (*Schistocerca* and *Melanoplus*) in which extraordinary chromosome numbers were encountered. Morgan ('15) has reported a case in *Phyllaphis* which differs from the above in that a considerable number of cells are affected rather than a few

isolated ones. In this particular instance all the cells of an entire cyst (male) contained the double number of chromosomes, which were readily counted, as the cells were just concluding the first spermatocyte division. This case presents one important point of difference from all other examples of abnormal division in that "the chromosomes are only half as large as are those at the corresponding stage of the normal spermatocyte stage"—a very unusual abnormality indeed.

In the material which I have examined two instances of abnormal chromosome number have been found which resemble the one described by Morgan in that large numbers of cells are affected, *but* the chromosomes are *not* reduced in size. A description of these cases will now be given.

The first, and in some ways the less interesting, of these cases was found in a specimen of *Loxa florida* Van D. (Family *Pentatomidæ*), of which both testes were affected in exactly the same way and to the same extent. This particular abnormality was restricted to a single lobe of the testis which happens to be composed of cells of an unusually small size (see Bowen '22).[1] The earlier stages in the affected lobe seem to be normal in every way (Fig. 4), but with the inauguration of the growth period of the primary spermatocytes a very unusual phenomenon sets in. Large numbers (but not all) of the cells in each cyst fuse together in pairs to form giant, bi-nucleate cells (Fig. 5). The cytoplasmic masses of the two cells seem to fuse completely, but the nuclei remain separate although they frequently become so closely appressed as to show little visible separation. As the growth period proceeds, this process of fusion goes on progressively, though somewhat irregularly, with the result that eventually the prophase cysts contain a heterogeneous mass of cells possessing in individual cases anywhere from one to eight or more nuclei (Fig. 6), each with its own complement of chromosomes.[2] The cells round out and become somewhat separated from each other, those containing numerous nuclei being roughly spherical in shape and of

[1] I have been able to examine only a single specimen of this species. It would be interesting to find out whether this abnormality has any relation to the sizes of the cells which might possibly cause its production repeatedly.

[2] In *Loxa* the spindle of the first spermatocyte division normally has eight chromosomes, as in a number of other pentatomids (*e.g., Euschistus*).

extraordinary size. The nuclei themselves become more or less lobulated during the growth period and so are difficult to separate and count, but the grouping of the late prophase chromosomes shows that each nucleus retains its identity and that the fusion process involves only the cytoplasms of the uniting cells. The remarkable fact about these giant cells is that they seem to possess only the single set of centrioles characteristic of a normal cell. Often these are not diametrically opposite each other (as is customary in Hemiptera), and as the spindle is formed for the first maturation division it tends to develop toward one side of the cell, becoming later on symmetrically located. How the centrioles of each of the cells represented in one of these giant spermatocytes are reduced to a normal number is not known; possibly the loss occurs before the fusion, which might itself be dependent on such an abnormal condition. Further, the centrioles which do take part in the formation of the spindle, are not unusually large, as one might anticipate, but are in fact no larger than the normal ones, at least not conspicuously so. Spindles are normally developed, and the metaphase chromosome plates are formed in a characteristic manner (though slightly irregular in cells with large numbers of chromosomes). I have been able to count cases with 16 and about 32 chromosomes, the higher numbers being less satisfactory for counting. I have, however, counted considerably more than 60 chromosomes in a single plate. Second maturation spindles seem to be normally formed (except, of course, with respect to chromosome number) and spermatids of a variety of sizes result, each spermatid being formed on a large scale but in an otherwise normal fashion. Whether the giant sperms resulting from the multinucleate spermatocytes reach maturity was not definitely ascertained, but at least some of them seemed to be undergoing the earlier stages of normal differentiation.

The origin of this abnormality is obscure. However, the apparent fusion (?) of cells to form multinucleate masses is not altogether unknown, as such aggregates have been noticed by Wodsedalek[1] in a number of mammals, particularly the ground

[1] Only a very brief abstract of this author's results has been available; see Abstracts of Papers, American Society of Zoölogists, Nineteenth Annual Meeting, December, 1921.

squirrel (*Citellus*). In the last-mentioned case, the spermatids also are multinucleate, which would seem to preclude the possibility of their forming normal sperms—a point in which this case is quite different from the one described in *Loxa*.

The second instance of abnormal divisions falling under *Case 2* presents in many ways the most interesting features of all. It is clear that aside from the method just described of multiplying chromosome numbers by cell fusion, the same result might be produced by some failure in spermatogonial division, such that the daughter chromosomes would be incorporated in a single nucleus, while the extra set of centrioles in some way became suppressed or lost. Such might conceivably have been the genesis of the case described by Morgan ('15), and of various triploid and tetraploid 'mutations.' The case now to be described seems to belong in this category. It was found in a specimen of *Euschistus variolarius* P. B. (Family *Pentatomidæ*), which had been collected in the fall of 1918 by Professor Wilson (without of course, any suspicion that it was abnormal), and kept in the laboratory during the winter. Upon my return to Columbia in February, 1919, this bug, together with other specimens of *Euschistus*, was very kindly turned over to me by Professor Wilson. The abnormality was found in only one specimen (No. 94) (of those sectioned), and in only *one* testis of that specimen. The other testis was normal in respect to this particular irregularity, though both presented other abnormal features. Whether the stay in the laboratory under somewhat unfavorable conditions was in any way responsible for the abnormalities is by no means clear; but from the fundamental nature of the particular abnormality here to be considered, it would seem more probable that it arose during one of the earlier instars and was quite uninfluenced by the abnormal environment of the adult. The material happens to have been fixed in Benda's Flemming (with the omission of the mordanting customary in the Benda method itself), and stained in Fe-hematoxylin. This method, intended primarily for the demonstration of the mitochondria, is not entirely satisfactory for the study of chromatic features; but the preparations proved entirely adequate for the study of most of the necessary details.

This particular abnormality consists of a tetraploid chromosome

number in most of the cells of one of the large-celled lobes (lobe No. 4—Bowen, '22) of one testis. There are six lobes in the testis of *Euschistus* (Bowen, '22), and in this particular testis I have been able to make counts of spermatogonial metaphase chromosome plates in five of the lobes. In four of these (lobes No. 2, 3, 5 and 6—Bowen, '22) the number of chromosomes is 14 (normal for the genus (Montgomery, '11)), and it is clear from other evidence that the one lobe (No. 1) in which no spermatogonial divisions could be counted, is likewise normal in chromosome number. In one lobe (No. 4), however, the chromosomes are very clearly abnormal in number as could be readily told from numerous metaphase plates. The exact number was not easily arrived at because the plates tend to be a trifle irregular, but in one or two particularly good cases I have been able to count 28 chromosomes with satisfactory clearness (Fig. 7). In adjacent cysts of spermatogonia in which the nuclei are in early prophase stages it can also be easily seen that the chromosomes are present in abnormal number, though exact counts were of course impossible. In the growth stages of the spermatocytes and in the spermatid stages the double number of chromosomes can be surmised from the unusually large size of the cells, which increase is shared by the cytoplasm as well as the nucleus. The tetraploid condition is, therefore, present throughout the entire lobe, though not exclusively, for I was able to find an occasional cyst (growth period and spermatid) in which, judging from the comparative sizes of the cells, the chromosomes were present in the normal number. These facts lead me to suppose that an irregular division of a germ cell at some very early stage gave rise to an abnormal series of spermatogonial cells which formed the bulk of the cells in this particular lobe. Among these, however, were included a few normal cells which evidently gave rise to the normal cysts found scattered infrequently among the abnormal ones.

Analysis of the constitution of the metaphase spermatogonial plates with 28 chromosomes should bear out the view that the tetraploid chromosome number represents a simple doubling of the usual diploid group. Unfortunately, the chromosomes in *Euschistus* are not of strikingly different sizes, but in the case of the *Y* chromosome the difference is sufficiently marked to be read-

ily distinguished; and as examination of Fig. 7 will show, there are two *Y* chromosomes (the very small ones) instead of the usual one (compare Montgomery, '11, Fig. 2). It is, I think, fair to assume that the other chromosomes are similarly represented in a duplex manner, an assumption which accords with the view now generally accepted of the nature of tetraploid chromosome groups.

Synapsis is apparently accomplished in a normal manner, and after the "diplotene" threads have become spread throughout the nucleus it is possible, in favorable places, to see that the threads are arranged in *pairs* and not in *quartets* as one might conceivably expect. In other words the homologous chromosomes are paired off just as they would have been in the presence of the diploid chromosome number, except that in each tetraploid nucleus there are two similar pairs instead of one. During the so-called confused period, when the chromatin threads become indistinct, the sex chromosome nucleoli stand out with great clearness. We should expect in a tetraploid nucleus four such nucleoli (barring possible fusions such as sometimes normally occur), representing two *X* (larger) and two *Y* (smaller) chromosomes. This expectation is exactly realized (Fig. 8), another bit of evidence which tends to prove that we are dealing here with a case of true tetraploidy.

When the chromosome tetrads condense in the prophases of the first maturation division, they resemble in every way the tetrads of the normal spermatocytes. (Compare Fig. 9 with Montgomery ('11), Figs. 86, 88 and 93.) It is clear beyond question that homologous chromosomes have paired off in a perfectly normal manner. These tetrads become condensed in the usual way to form the definitive chromosomes of the first maturation division, and the spindle of this division, when seen in side view (Fig. 10), presents no unusual features (except of course for the chromosome number). There happened to be one cyst of cells in various phases of the first spermatocyte division and these dividing cells have been carefully studied. I have been able to find nothing in the manner of spindle formation or chromosome division in any way abnormal, except that the plates have the diploid rather than the haploid number of chromosomes. This

point was studied with special care. Two metaphase plates in polar view were found in which the chromosomes could be readily counted, although both were a trifle irregular in arrangement. One of these plates (Fig. 11) seemed to be in the final stage of formation, and some of the chromosomes were still not completely oriented. Fortunately this was particularly true of the *Y* chromocomes, whose constitution was thus rigorously demonstrated. In the normal cells the *Y* chromosome is represented by a dyad which is divided in the first spermatocyte division (see Montgomery, '11, Fig. 94). In a tetraploid cell we should expect to find two such dyads, and this is actually found to be the case. The *Y* chromosomes have not conjugated, but each has behaved exactly as though the other were absent. The parts of the dyad have been in each case precociously separated (Fig. 11). Furthermore, two unusually large chromosomes can be readily picked out (*A* and *A'*), and these seem to correspond to the single large chromosome of the normal haploid plate (see Wilson, '05*a*, Fig. 3*a*). A count of all the chromosomes (counting the *Y* dyads each as a single chromosome) gives a total of 17—one more than the expected number (16, or 8 times 2). It seems likely that the extra chromosome may be due to the counting of an *X* dyad, the parts of which have become separated, as two chromosomes instead of a single one. In Fig. 12 another plate is given which, judging from its neighbors, is probably in a very early anaphase stage. The plate is somewhat more compact, and the *Y* chromosomes, now presumably being drawn apart, are seen as single chromosomes. In this plate 16 chromosomes are clearly to be counted, although one of them is irregularly placed.

Unfortunately no second maturation divisions were found, so that it was impossible to check up the behavior of the *X* and *Y* chromosomes in that division. The spermatids seem to be normal in every way, except that they are larger than is usual; and at least the earlier stages of differentiation are gone through in a normal manner. Subsequently, some of them certainly become abnormal, but so do some of the sperm in an adjacent lobe (No. 6); and it is, therefore, impossible to tell whether the degeneration is connected with the tetraploid condition or not.

CASE 3.

The occurrence of divisions with abnormal numbers of both chromosomes and centrioles is of common occurrence in the maturation divisions in Hemiptera (see for example Henking, '91), and such divisions have also been described in spermatogonia (Montgomery, '98), and in cells from the connective tissue of the testis (Paulmier, '99). The general features of these divisions are well known, and they probably originate from abnormal spermatogonial divisions in which the daughter chromosome groups have failed to produce separate nuclei. In the cases here noted, from *Chlorochroa uhleri* (=*persimilis*) Stål (Family *Pentatomidæ*), large numbers of cells were affected particularly along one half of one testicular lobe, and to a lesser degree the contiguous portion of an adjacent lobe. In some cysts, as could be told from the unusual size of the nuclei with extra chromosomes, large numbers of spermatocytes (and spermatids) were affected, while in others all the cells were normal. The causative agent operative in this case would accordingly seem to differ from the more or less accidental sources of abnormal division figures. The chief interest of this case lies in the fact that the material had been prepared to demonstrate the Golgi apparatus, and it was accordingly possible to study the distribution of the dictyosomes (fragments of the Golgi apparatus) in relation to the multipolar spindle.

Numerous cases of tripolar[1] spindles in the first maturation division were found at both the metaphase (Fig. 2) and late anaphase (Fig. 1) stages, of which two are figured. As I have shown in another place (Bowen, '20), the dictyosomes collect (in equal amounts) around the ends of the normal (bipolar) spindle at the beginning of the metaphase, a position which they maintain during the anaphase, the Golgi material being thus distributed with approximate equality to the daughter cells. I suggested that the centrioles represent the morphological foci of the influences which bring about this equal distribution of the Golgi material.

[1] There were also numerous cases in which the centriole number was normal, but with abnormal chromosome numbers, as in Case 2; but as these presented no points of special interest, they are not here considered. The distribution of the dictyosomes was in accordance with the expectation for a bipolar spindle.

If this suggestion be correct, in tripolar spindles the dictyosomes should obviously be arranged around each spindle pole (centriole) in a manner similar to that found in bipolar figures. This, is, in fact, the case as the figures show very clearly (Figs. 1 and 2), and seems to contribute additional evidence to that already accumulated tending to demonstrate an element of unusual regularity in the distribution of the Golgi apparatus in cell division.

Discussion.

Abnormal mitoses in the male germ cell cycle are chiefly of interest from the standpoint of the sperms which might be produced as a result of such divisions. It is clear that the sperms derived from divisions belonging to *Case 1* would always be abnormal; and those of *Case 3* (if the division were ever completed, which seems doubtful[1]) would likewise be abnormal in the great majority of cases. Whether any such abnormal sperms ever take part in successful fertilization is questionable. After divisions of the *Case 2* variety, however, there is no reason for supposing that the resultant sperms might not behave in an entirely normal manner in fertilization. This is particularly true of the case which I have described in *Euschistus*, and to the possibilities there presented I should like to give particular attention.

It is evident that tetraploid spermatogonia would probably give rise to diploid primary spermatocytes, and this probability has been definitely proved in the *Euschistus* case. It seems equally probable that the resultant sperms would likewise contain a group of chromosomes in the diploid rather than the normal, haploid number. Unfortunately definite proof of this has not been obtainable, but everything indicates that such is actually the case. If such diploid sperms should fertilize a normal (haploid) egg, a triploid individual would result; and if, as an almost impossibly rare coincidence, the egg should also be diploid, through some abnormality, a tetraploid individual would result. The triploid individuals might be numerous due to the involvement of a large number of sperms, but they would be accompanied by diploid individuals derived through some of the normal, haploid sperms

[1] It is perhaps more probable that these abnormal mitoses fail, giving rise to the familiar giant spermatids with one large nucleus but a multiple set of centrioles.

which are produced by the normal portions of the testis. It is interesting to note that Bridges ('21) has recently reported a case of triploidy in *Drosophila* which exactly fulfills these expectations. The egg rather than the sperm, as could be determined from genetic evidence, happens, however, to have been the gamete affected, but the arrangement of the ovary is such that the difference in sex does not affect the applicability of the argument. The facts which I have been able to make out in *Euschistus* prove (so far as they go) that the presence of the chromosomes in the tetraploid number, following some irregularity of unknown origin, in no way affects the normal progress of spermatogenesis[1] (and presumably of oögenesis as well). This is, I believe, the first cytological evidence that abnormal germ cells, once established, may thereafter proceed in an entirely normal manner—probably with the ultimate production of functional sperms (or eggs).

These facts have a very interesting bearing on the origin of triploid and tetraploid individuals, a matter in regard to which opinion is at present in a very unsettled state. It has been held by Gates ('09), with the subsequent assent of Strasburger, that the doubling of the chromosome number (tetraploid individuals) might arise "as the result of a suspended mitosis in the fertilized egg or in an early division of the young embryo." Stomps ('12), on the other hand, has suggested that tetraploid individuals may result from the union of two diploid gametes, triploid individuals being obviously formed on this hypothesis by the union of a haploid with a diploid gamete.[2] Further, the nature of the chromosome group in a diploid gamete is again open to a difference of opinion, since it is evident that the diploid number may have arisen from a normal (diploid) auxocyte through failure of

[1] Provided of course that other disturbing elements such as multiple centrioles are absent.

[2] The possibilities of polyspermy have also been considered by Gates particularly in cases of triploidy, but our knowledge of this condition in animals lends no support to the belief that it is ever a factor in the production of triploid or tetraploid individuals. Further, both these authors were interested almost exclusively in plants (in which (*Œnothera*, for example) triploid and tetraploid individuals have actually arisen under observation), in which further special possibilities (apogamy, for example) have to be considered that are not met with in animals. These matters need not be considered further in this paper.

reduction, or from a tetraploid auxocyte after normal reduction has occurred.

My observations indicate that the requisite conditions for the origin of the triploid, and perhaps tetraploid, individuals may be fulfilled by a combination of the possibilities suggested by the hypotheses outlined above. Thus, it seems certain in the *Euschistus* case that a suspended (or otherwise abnormal[1]) mitosis has occurred during some early division in the germ cells, giving rise to a tetraploid condition confined to the descendants of the particular cell involved. If, as seems probable, each cyst of spermatogonia is derived by the repeated division of a *single* cell derived from an early germ cell, the occurrence of the tetraploid cysts would be accounted for, and also the diploid cysts produced concurrently from the early germ cells of normal constitution. The spermatocytes descended from the tetraploid cells have undergone a *reduction* in the proper sense of the term, but the *number* of chromosomes in the resultant gametes is undiminished ("unreduced") as compared with the number in the normal (haploid) ones. The fusion of such a diploid gamete with a normal one would obviously produce a triploid individual. An explanation along these lines seems to me preferable to the one sometimes given which explains triploidy as due to the union of a normal and an unreduced (properly speaking) gamete; for while such unreduced gametes have been supposed to arise (in Hemiptera) through the suppression of one (or both) of the maturation divisions, they always contain an abnormal number of centrioles (as Paulmier, '99, long ago showed), and the weight of evidence is against the probability of their functioning normally in fertilization.

The origin of tetraploid individuals by this method would depend upon an exceedingly rare coincidence, and, as a matter of fact, tetraploidy in animals seems to be a rather rare phenomenon.

[1] The case of *Loxa* suggests the possibility that a tetraploid germ cell might arise from the fusion of two normal ones. By analogy such a fusion product might well contain the normal centriole content, as observed in *Euschistus*. The usual explanations advanced to account for the doubling of chromosomes all fail to account for the observed normal number of centrioles—an important part of the story, which has not yet received adequate attention. In this connection it is of interest to note that centrioles are generally absent in the higher plants.

In plants, on the other hand, it occurs more commonly—in *Œnothera* for example, where the repeated occurrence of unusual chromosome complexes would perhaps indicate that some unstable condition exists which would increase the probability of a coincident production of diploid gametes of both sexes.

The viewpoint which I have here tried to develop may be summarized in the following way. A normal, diploid zygote is formed by the union of two haploid gametes.[1] After a number of divisions the germ cells (one or more?) are segregated, and these then multiply to form the primordial germ cells and subsequently spermatogonia (or oögonia). An abnormal division at some early stage resulting in a doubling of the chromosomes might conceivably give rise to a gonad composed entirely of tetraploid cells—a condition never yet observed. A similar abnormal division in one of the primordial germ cells would result in many cysts of tetraploid spermatogonia (or oögonia), as I have found in the *Euschistus* case—certainly a rare phenomenon or it would have been long since reported. Proceeding still further, a similar abnormal division at the time a cyst of spermatogonia (or oögonia) is begun, would give rise to a single cyst of tetraploid cells (see the case reported by Morgan, '15), while abnormal divisions at a subsequent time might give rise to one or several tetraploid cells among many normal ones in the same cyst. The tetraploid cells thus produced undergo normal *synapsis* and *reduction*, but the chromosome number, being thus diminished only by the usual one half, is diploid in the resulting gametes. The union of such a diploid gamete with a normal (haploid) one produces a triploid individual, while the union of two such diploid gametes (due to a very rare coincidence) produces a tetraploid individual.

In conclusion, it may be noted that the behavior of the chromosomes in the *Euschistus* case adds further evidence to that already accumulated tending to demonstrate the genetic continuity of the chromosomes. In the same testis there are (normal) diploid and (abnormal) tetraploid spermatogonia, each of which gives

[1] It is conceivable that, as Gates suggests, the first (or other early) cleavage might be suppressed, resulting in a tetraploid individual, but there is no evidence among animals that this ever occurs, and the difficulties in the way of such an explanation are great.

rise to the expected haploid and diploid chromosome complexes in the maturation divisions. The fact that in synapsis (and subsequent stages) the homologous pairs of chromosomes behave exactly as though they were present in the diploid number, is another fact of great theoretical significance. This last-mentioned point is of particular interest because of the condition recently reported in a triploid specimen of *Canna* by Belling ('21). This author finds that in the pollen-mother-cells there are nine *triads* (the haploid number is nine), "each of which separates into two and one on the spindle, in a random manner with regard to the two poles." In the tetraploid individual of *Euschistus*, on the other hand, no indication of a tendency to form multiple groupings of this kind has been observed at any stage.

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EXPLANATION OF PLATES.

All of the figures have been outlined as far as possible with the camera lucida at an initial enlargement of approximately 3,800 diameters. The outlines have been extensively corrected and details added free hand. In reproducing, the figures have been reduced uniformly to an enlargement of approximately 3,000 diameters. In every case the method employed in the preparation of the original object has been indicated.

P, plasmosome; *X* and *Y*, idiochromosomes.

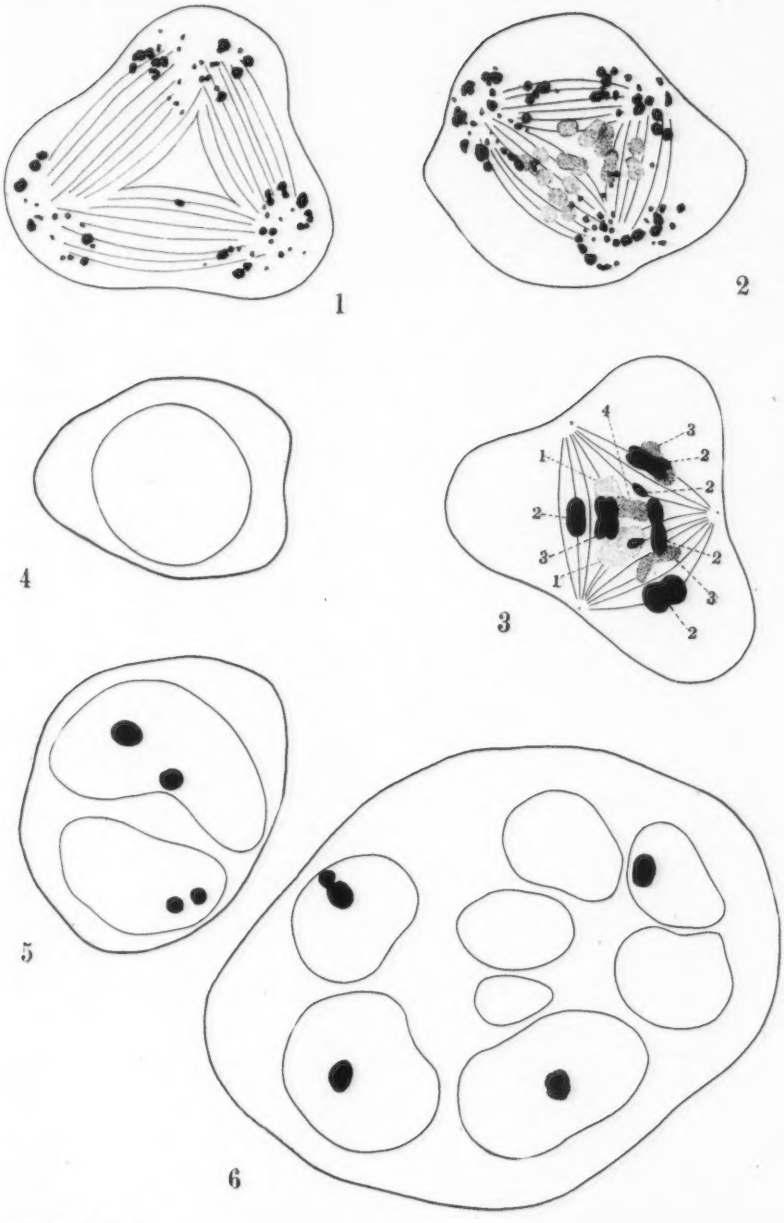
PLATE I.

FIGS. 1 and 2 are from *Chlorochroa uhleri;* Fig. 3 is from *Chelinidea vittiger;* Figs. 4, 5 and 6 are from *Loxa florida.* With the exception of 1 and 2, the original preparations were from material fixed in Flemming and stained with Fe-hematoxylin.

FIGS. 1 and 2. Tripolar spindles in the first spermatocyte divison. Some of the chromosomes (stippled) are indicated in Fig. 2. (Osmic impregnation.)

FIG. 3. Tripolar spindle with normal number of chromosomes in the first spermatocyte division.

FIGS. 4, 5 and 6. Primary spermatocytes in the later diplotene stage and in the earlier and later growth period, showing progressive fusion of the cytosomes.


1
2
4
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5
6

PLATE II.

All the figures are from *Euschistus variolarius*. The original preparation was from material fixed in Benda's Flemming and stained with Fe-hematoxylin.

FIG. 7. Spermatogonial division (polar view) showing metaphase plate with 28 chromosomes.

FIG. 8. Primary spermatocyte in the later growth period.

FIG. 9. Single tetrad from a tetraploid spermatocyte nucleus in the late prophase.

FIG. 10. First spermatocyte divison; lateral view.

FIGS. 11 and 12. Early and late stages in the metaphase (approximately) of the first spermatocyte divison; polar view.

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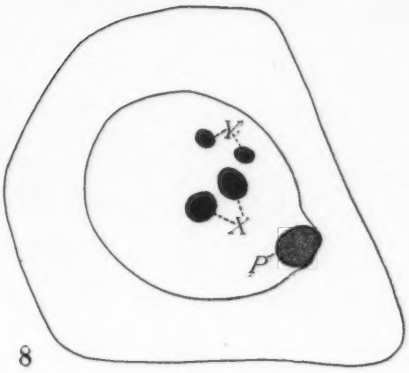

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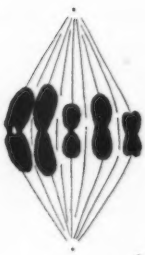

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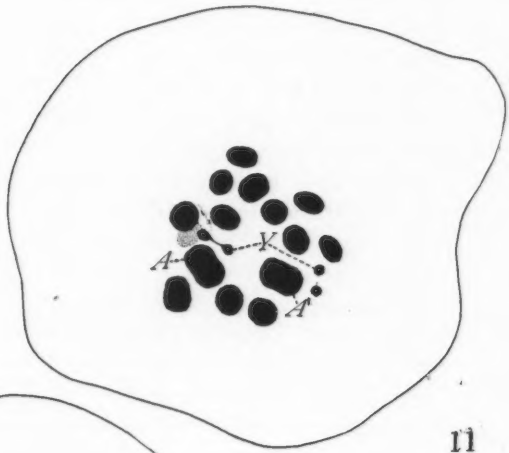

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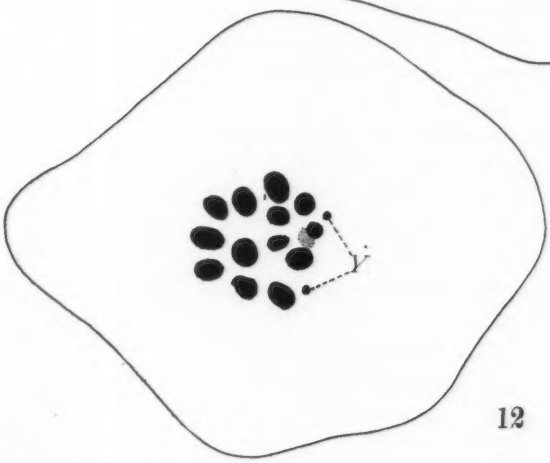

12

RELATION BETWEEN INTENSITY OF LIGHT AND RATE OF LOCOMOTION IN *PHACUS PLEURONECTES* AND *EUGLENA GRACILIS* AND ITS BEARING ON ORIENTATION.

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INTRODUCTION.

According to the De Candolle-Verworn theory of orientation which has been widely accepted, the action of the locomotor appendages in organisms is dependent upon the intensity of the light received by the receptors connected with them; so that if one side of a bilaterally symmetrical organism, *Volvox, e.g.*, is more highly illuminated than the other the locomotor appendages on one side beat more effectively than those on the opposite side, resulting in turning until the two sides are equally illuminated and the organism is oriented. Torrey ('13), Bancroft ('13), Loeb ('18). and others maintain that orientation in asymmetrical organisms like *Phacus* and *Euglena* is in principle precisely the same as orientation in bilaterally symmetrical organisms. These organisms rotate on their longitudinal axes and proceed on a spiral course with a given surface continuously directed outward. The locomotor appendages are, consequently, on one side when the organism is in one position on the spiral and on the opposite side when it is in another position. Owing to this it is held by those mentioned above, that orientation in these asymmetrical organisms is essentially like that in bilaterally symmetrical organisms. If this contention holds it is evident that the rate of locomotion should depend upon the luminous intensity of the field. With this in mind we have investigated the relation between the intensity of light and the rate of locomotion in *Phacus* and *Euglena*.

Phacus pleuronectes.

Phacus is a small green flagellate somewhat like *Euglena* in structure and behavior. The species studied closely resembles

Phacus pleuronectes (Müller). It was kept in the laboratory in fairly good condition for several months. All of the specimens used in these observations were taken from the same culture jar. The experiments were all carried out in a large dark room. The apparatus used was arranged as follows: A rectangular box 180 cm. long, 15 cm. wide and 15 cm. deep, open at one end and the top, was placed on a table with a microscope near the open end. An electric lamp in the box was attached to the under surface of a short board which fitted into grooves on the top of the box, and could be moved back and forth so as to rapidly change the luminous intensity at the microscope. The lamp was so adjusted and screened that it produced a horizontal beam of light which crossed the stage of the microscope. The beam of light, before reaching the organisms, passed through 3 cm. of water in a glass container. Thus the longer waves from the lamp were absorbed by the water and any possible effect of heat on the behavior of the organisms was greatly reduced, if not entirely eliminated.

All of the observations were made under the low power of a compound microscope with the organisms on the stage in an aquarium, about 3 cm. square, constructed of the best plate glass obtainable. The eye-piece of the microscope contained a micrometer scale which divided the field into equal divisions, each .017 mm. long.

In making the observations, 30 to 40 specimens of *Phacus* were taken from a culture jar and put into the aquarium, which contained clear water about 1 cm. deep taken from the same jar. The aquarium was then put into the beam of light on the stage of the microscope and the distance from the lamp adjusted so as to produce the luminous intensity desired. An individual which was accurately oriented was now selected and carefully observed as it proceeded toward the light. (All of the observations were made on positive individuals.) When the anterior end reached a cross-bar of the micrometer scale the stop-watch was started, and when it reached the cross-bar 20 divisions, .34 mm., beyond, the watch was suddenly stopped and the time consumed recorded. The intensity was then suddenly raised or lowered and the same individual again timed, in the manner just described. Thus there were two readings made on each individual. In some cases the readings were made first in the higher, and in other cases first in

the lower intensity. When the intensity was increased there was ordinarily no shock-reaction, and the organisms were timed immediately after the intensity was changed, *i.e.*, shortly after the close of the test in the lower intensity. But when it was decreased there usually was a shock-reaction, and if this occurred, the reading was not started until after the organisms had fully recovered which usually required only a few moments. The intervals preceding the readings in the lower intensities were, however, always somewhat longer than those preceding the readings in the higher intensities.

The results obtained are presented in Tables I. and II. Table

TABLE I.

RELATION BETWEEN INTENSITY OF LIGHT AND RATE OF LOCOMOTION IN *Phacus pleuronectes* (POSITIVE INDIVIDUALS).

Each line contains two readings for one individual in different intensities of light; the last line, the averages for all 20 individuals. The record in the lower intensity was obtained first in each instance.

Designation of individuals.	Time, in seconds, required to travel .34 mm. in an intensity of 1023 m.c.	Time, in seconds required to travel .34 mm. in an intensity of 4128 m.c.
1	5.7	6.2
2	5.4	5.8
3	6.7	7.4
4	5.5	5.2
5	6.4	7.0
6	7.0	7.5
7	6.2	6.2
8	7.0	7.2
9	4.9	4.4
10	6.0	5.7
11	5.5	5.4
12	5.4	5.3
13	5.4	5.3
14	5.0	4.7
15	5.2	5.2
16	4.8	4.8
17	5.0	5.2
18	4.7	4.9
19	5.2	5.0
20	5.7	5.5
Average	5.63	5.69

I. includes, for each of 20 individuals, two records, one taken in higher and the other in lower intensity. In each case the record for the lower intensity was obtained first. That is, the rate of locomotion for a given individual was ascertained in the lower intensity, then the light was moved nearer to the microscope and the rate ascertained for the same individual in the higher intensity. After the two readings had been obtained for one individual, the light was moved to its original position, another individual timed in the same way, and so on until the rates for 20 or more individuals were recorded.

By referring to Table I., it will be seen that the rate of locomotion of any one individual in the lower intensity was practically the same as it was in the higher intensity. The table shows that the average time required to travel .34 mm. was 5.63 sec. in the lower and 5.69 sec. in the higher intensity, indicating a slightly higher rate in the lower intensity. The difference referred to is, however, only .06 of a sec. This is probably within the limits of error, since a further examination of the table shows that the rate was not consistently higher in the lower intensity, 9 individuals traveling faster in the higher intensity, 8 slower and 3 at the same rate in the two intensities. That is, the rate varied about as frequently in one direction as in the other. This shows that there is, in *Phacus*, practically no difference in the rate of locomotion in luminous intensities varying from 1023 m.c. to 4128 m.c., and it indicates that locomotion is not to any considerable extent immediately related with the intensity of the illumination.

A summary of the results obtained in all of the observations made are presented in Table II. By referring to this table it will be seen that the rate of locomotion was on the average slightly higher in the lower intensities than it was in the higher, the average time required to travel 0.34 mm. being 6.002 seconds in the former and 6.134 seconds in the latter. The difference is, however, so small and inconsistent, being in favor of the higher illumination in two out of the nine sets of tests, that its significance is questionable. These results support the conclusions formulated above. They show that the rate of locomotion in *Phacus* is within wide ranges, practically independent of the intensity of

light when exposed for short periods of time, and that if light has any immediate effect on the rate, it has a retarding effect.

TABLE II.

RELATION BETWEEN INTENSITY OF LIGHT AND RATE OF MOVEMENT IN *Phacus pleuronectes* (POSITIVE INDIVIDUALS).

Records in the higher intensity obtained first in the first three sets of experiments and last in the rest.

Each line gives the average rate, for a set of individuals, in a higher and a lower intensity. Each individual represented in the averages was timed twice, once in a higher, and once in a lower intensity. The + signs in the last column indicate a higher rate in higher intensity, the — signs a lower rate in the higher intensity.

Number of Individuals.	Intensity in m.c.	Average Time, in Seconds, Required to Travel .34 mm.	Intensity in m.c.	Average Time, in Seconds, Required to Travel .34 mm.	Difference between the Time Required in the Two Intensities.
15	91	5.71	325	5.73	−.02
20	106	6.90	325	6.55	+.35
20	58	6.58	325	6.96	−.38
12	91	5.26	325	5.36	−.1
13	31	5.91	325	5.82	+.09
20	31	5.78	325	6.01	−.23
20	16	5.44	325	6.05	−.61
20	1,032	5.63	4,128	5.69	−.06
18	459	6.81	4,128	7.04	−.13
Total average		6.002		6.134	

Euglena gracilis.

The observations on *Euglena* were all made on one species (*gracilis*). The specimens used were obtained in laboratory cultures containing wheat, a substance which is very favorable for the growth of this species. The methods employed were precisely the same as those employed in the study of *Phacus* with the following exceptions: The observations extended over a wider range of intensities; they were made under a binocular in place of a compound microscope; and each individual was tested successively in four different intensities beginning in every case with the lowest, in place of in two different intensities beginning sometimes in the higher and sometimes in the lower; and the course was 0.74 mm. in place of 0.34 mm. long.

A summary of the results obtained is presented in Table III.

TABLE III.

RELATION BETWEEN RATE OF LOCOMOTION AND LUMINOUS INTENSITY IN *Euglena gracilis.*

Each individual was timed successively, once in each of the intensities indicated, beginning in every instance with the lowest. The time presented is, in every case, the average of that required for each of the individuals indicated to travel once over the course in the illumination indicated.

Date of Observation.	No. of Individuals.	Time, in Sec., Required to Travel 0.74 mm. in an Intensity of			
		286 m.c.	459 m.c.	853 m.c.	2106 m.c.
Jan. 19...	8	5.88	6.05	5.53	5.12
Jan. 28...	2	5.15	5.6	5.65	5.75
Jan. 28...	2	6.1	6.5	5.55	5.95
Feb. 1....	2	4.7	4.7	3.6	4.9
Feb. 1....	8	7.47	7.55	7.91	7.64
Feb. 6....	8	6.01	5.36	5.17	5.27
Feb. 7....	26	5.7	5.36	5.30	5.45
	Average....	5.858	5.873	5.53	5.725
		84 m.c.	142 m.c.	286 m.c.	853 m.c.
Feb. 8....	2	5.85	6.1	5.9	5.5
Feb. 12...	7	8.8	8.45	8.82	8.73
	Average....	7.325	7.275	7.36	7.115

This table shows that in intensities ranging in the one set of experiments from 286 m.c. to 2106 m.c. and in the other from 84 m.c. to 853 m.c. there is remarkably little difference in the rate of locomotion, the difference between the rate in the lowest intensity and that in the highest intensity being only .0276+ mm. per second in the one set and .0298+ mm. in the other. It shows that, in the time it takes *Euglena* to travel 10 mm. in the lowest intensity it would travel only a little more than 10.2 mm. in the highest intensity, and that the lowest rate is not consistently in the lowest intensity.

The results obtained consequently indicate that light in certain intensities slightly accelerates locomotion in *Euglena,* and slightly retards it in *Phacus.* Orientation cannot, however, be due to the effect on the rate of locomotion of difference in the illumination

of the sensitive tissue in different positions on the spiral course of these organisms, as is demanded by the De Candolle-Verworn theory applied to asymmetrical organisms by Torrey, Bancroft and others, for the effect on the rate is, even under the most favorable condition, so small that if orientation were dependent upon this, it would require much longer than it actually does; and moreover, orientation occurs under luminous conditions in which an increase or a decrease in intensity does not appear to appreciably effect the rate of locomotion.

The facts presented above have no bearing on the question as to the effect of light on the rate of locomotion in long exposures. All of the evidence obtained by various investigators in reference to this indicates that organisms like *Phacus* and *Euglena* come to rest if they are subjected for long periods to low illumination or darkness. This is probably owing to the effect of light on physiological processes (*e.g.*, photosynthesis) which in turn affect the activity of the organisms.

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DEDIFFERENTIATION IN ECHINUS LARVÆ, AND ITS RELATION TO METAMORPHOSIS.

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1. INTRODUCTION.

The following observations and experiments were made at the Marine Biological Association's Laboratory at Plymouth in July and August, 1920.

The accidental discovery that dedifferentiation, of a type similar to that already studied in *Clavellina*, occurred in Echinoderm larvæ led me to make further observations on this phenomenon. Owing to lack of material, they are very fragmentary. I hope to resume them at the earliest opportunity. Meanwhile their theoretical bearing on the question of metamorphosis warrants their publication in their present form.

I have to thank the Director and Staff of the Plymouth Laboratory for their assistance. The work was carried on with the aid of a grant from the Royal Society.

2. DIFFERENTIAL MODIFICATION OF GROWTH.

Experiments were started on fertilized eggs of *Echinus miliaris* in order to test certain conclusions of Child's ('16 and '17) as to

the effect of dilute poisons on differential modification of growth, and to compare the effects of cyanides and mercury salts.

A. When fertilized *Echinus* eggs were placed in KCN *n*/50,000[1] growth ceased at the 2- or 4-cell stage. A small percentage of eggs were cytolysed and many had multiple asters and irregular segmentation. In KCN *n*/100,000, after 24 hours more eggs were cytolysed, a certain proportion had got no further than the 2- or 4-cell stage, and a small percentage had become blastulæ, most of them of the abnormal solid type (stereoblastulæ). The controls had in the same period reached the gastrula stage.

After 72 hours, 24 hours after the controls had reached the early pluteus stage, these blastulæ had become late gastrulæ. These were transferred to sea-water, and developed as far as the pre-pluteus stage, but never became normal plutei, thus bearing out Child's conclusions that considerable poisoning in the early stages in some way disturbs the relations of parts so that even when the developing organism is replaced in normal conditions, it can only develop up to a certain stage, and no further.

In this connection the observations of Perkins ('02) are of interest. He discovered that the hydriform larva of *Gonionemus* when kept in the laboratory lost its typical form and assumed an irregular amœboid shape. It moved about, apparently ingested food, repeatedly underwent a form of fission, and lived for over 2 months.

Obviously, therefore, viability and capacity to develop are by no means synonymous, and we have the theoretical possibility of the existence of persistent larval forms due to unfavorable conditions as well as to genetic variations.

Other larvæ were replaced from KCN *n*/100,000 to sea-water at 48 hours, from the blastula stage. After a further 48 hours, many had formed early plutei. These were mostly of a very wide-angled type (Fig. 9), thus showing what Child finds in similar circumstances, and has called "differential recovery."

Thus between the 48th and the 72nd hour in the solution the larvæ had lost their power of recovery in sea-water, though not of continued existence.

[1] The molecular concentration is simply given for convenience: the actual ionic concentration of CN would naturally vary with the hydrogen-ion concentration and other factors of the sea-water used.

B. $HgCl_2$. *n*/5,000, *n*/10,000, *n*/20,000, *n*/50,000 and *n*/100,000 solutions allowed segmentation to proceed to the 2-, 4-, or 8-cell stage in 24 hours (mostly to the 4-cell stage). Many segmenting eggs, however, were obviously damaged, and by 48 hours all were dead. The course of events was similar in a *n*/200,000 solution, except that by 24 hours segmentation had proceeded to the 4- to 32-cell stage (mostly to the 8-cell stage). Transference from *n*/100,000 and *n*/200,000 to sea-water at 24 hours had no effect, all being dead at 48 hours. The dead blastomeres were well-preserved, not cytolysed or disintegrated.

As the solutions used were obviously too strong, 24-hour gastrulæ from the control were placed in *n*/500,000 and *n*/1,000,000 solutions. These too were all dead 24 hours later (48 hours from fertilization); but they showed the interesting phenomenon of differential disintegration. All the tissues except the archenteron had entirely disintegrated, and lay as a sheet of cell-débris on the bottom of the vessel. The archenteron, on the other hand, was well preserved, and the outlines of its walls could be clearly seen (Fig. 10). The spicules were usually to be seen adherent to it. Sometimes it appeared solid. This is obviously an occurrence of the nature of those observed by Child ('15), and utilized by him in his axial gradient theory.

3. Dedifferentiation of Larvæ.

Unfortunately no further *Echinus* were to be had, and I was therefore unable to repeat the experiments with more suitable strengths of solution. Wishing, however, to see what the effect of poisons might be on more advanced stages, I transferred some of the plutei from the controls to various solutions of KCN and $HgCl_2$. The most interesting results were obtained in the mercuric-chloride solutions.

C. A preliminary experiment was made with 2-day plutei in $HgCl_2$ *n*/1,250,000. After 6 hours, many showed a retraction of the arms, leaving part of the skeletal spicules protruding. After 24 hours, many had died. The following types could be distinguished:

1. Partially dead, with disintegration of the tissue of the arms; the aboral ectoderm not disintegrated, or with a few cells migrated

out of the tissue and adherent to it externally (these cells appeared normal, in contradistinction to the pathological granular disintegration of the tissue of the arms). The form of the gut was well preserved, and no disintegration of any sort was visible in it (*cf.* Section 2, *B*).

In some cases it appeared that all tissues had disintegrated with the exception of the somewhat contracted gut, which was thus left by itself; but I was unable to be quite sure of this.

2. Slightly shrunken plutei with the skeleton protruding through the arms; these latter might be from 25 to 75 per cent. of their original length.

3. As (2) but the skeleton not protruding from the arms. The terminal portions of the spicules had apparently been resorbed by the arms as they contracted.

All the plutei had sunk to the bottom, and ciliary action was so much reduced that only very sluggish movement was taking place.

In addition to the types mentioned, others were seen which were of spheroidal form, dense, without arms, and with simple or no spicules. I at first thought that these might be examples of extreme dedifferentiation, but on examining the control culture, I found at the bottom a certain proportion of quite similar organisms. The culture was slightly overcrowded and these were doubtless susceptible individuals inhibited by the slightly adverse conditions. These types are mentioned to draw attention to the necessity for careful control in similar experiments. (This has already been emphasized by Shearer, De Morgan and Fuchs ('13), p. 274, etc.) They are also interesting as indicating that considerable inherited variations in vigor and resistance occur among the offspring of a single pair of *Echinus*.

In the later experiments now to be recorded, the plutei were picked out from the control culture with a fine pipette under the binocular microscope, transferred to the medium to be tested and there examined, to see if any of these minute armless forms had been transferred by mistake. In no experimental vessel was more than one of these forms discovered immediately after transfer; it can therefore be safely assumed that in this series the experi-

mental animals were for practical purposes all healthy plutei with well-developed arms.

D. Four-day plutei transferred to *n*/3,000,000 and *n*/4,000,000 $HgCl_2$. After 24 hours, all appeared normal, and about 90 per cent. were still swimming. After 48 hours, all had sunk to the bottom, ciliary action was somewhat reduced, there was a slight general shrinkage and loss of transparency as compared with the controls, and the arms had become slightly though definitely shorter, but without protrusion of the skeleton. The experiment was here discontinued, after another series had been started.

E. Five-day plutei (*a*) to $HgCl_2$ *n*/1,000,000, (*b*) to $HgCl_2$ *n*/2,000,000, (*c*) control in same amount of outside sea-water.

The controls remained healthy, with long arms, throughout the first 4 days of the experiment. The results may be tabulated as follows:

TABLE I.

	(*a*) $\frac{n}{1,000,000}$.	(*b*) $\frac{n}{2,000,000}$.
24 hrs.	All at bottom. Marked arm-resorption; no protrusion of spicules; arms mere knobs or ¼ to ½ normal length; aboral end usually swollen.	All at bottom. Moderate arm-resorption; no protrusion of spicules.
48 hrs.	Still further arm-resorption.	Slightly more resorption than at 24 hours.
72 hrs.	Arms absent or only knobs; a few with total dedifferentiation of oral end; many with aboral end no longer swollen, mesenchyme clumped, gut shrunken, spicules sometimes just protruding.	Slightly more resorption than at 48 hours.
96 hrs.	Spicules often protruding, most showing disintegration at oral end, and narrow form of body.	Condition as in (*a*) after 24 hours.
120 hrs.	Dead.	Condition as in (*a*) after 48 hours.
144 hrs.		Condition as in (*a*) after 96 hours.
168 hrs.		Dead.

(*a*) *n*/1,000,000. Some individuals after 24 hours in the medium are represented in Fig. 1.

The controls had arms about 25 per cent. longer than Fig. 1, *c*. It will be seen that in all cases the arms have diminished in length. They have also become more dense in appearance, being in the more advanced examples quite opaque. The diminution is rarely less than that seen in Fig. 1, *c*, and ranges from about 20 per cent. to about 80 per cent. The skeleton does not protrude at the tips of the arms, and the missing parts have apparently been resorbed. Another marked feature in the large majority of the specimens is the dilatation of the trunk and aboral regions (Fig. 1, *a–c*), which leads to a bulgy form, usually with a wide separation of the aboral ends of the spicules.

This may be explained as an effect of differential susceptibility, the more susceptible oral region contracting before the aboral, which then becomes distended with fluid. In some specimens (Fig. 1, *c*) the trunk ectoderm was seen to be definitely thinner than normal, implying distention. Other specimens (Fig. 1, *d* and *e*) did not show these phenomena at all markedly; in every case, such non-distended individuals exhibited extreme arm-resorption, and were also well below the average size. These were presumably individuals of general low susceptibility, in which all regions had suffered, the aboral ectoderm also being contracted, and the total volume of body-fluid being in some manner diminished. In point of fact, they were usually seen (Fig. 1, *d*) to have abnormally thick aboral and trunk ectoderm.

The number of pigment-granules appeared in general to have increased.

The appearances after 48 hours were very similar, with an increase in the amount of arm-resorption, and of aboral contraction.

After 72 hours, none had more than quite vestigial arms (Fig. 2, *a*), while the majority were almost or quite armless (Fig. 2, *c*). Very few now showed the separation of the aboral ends of the spicules, and not so many the swelling of the trunk region (Fig. 2, *c*). Many, on the contrary, were very narrow, especially at the aboral pole, and showed a condensation of the mesenchyme cells to form clumps (Fig. 2, *a*). In Fig. 2, *b*, the aboral region of the same specimen is shown under a higher power. The large clumps of mesenchyme cells are extremely like the clumps of

blood (mesenchyme) cells seen in dedifferentiated specimens of the Ascidian *Clavellina* (*cf.* Driesch, '06).

Up till 48 hours, the gut had remained apparently normal. In these specimens, however, though the stomach was of normal (Fig. 2, *a*) or even more than normal size (Fig. 2, *c*) the œsophagus and intestine showed some degree of contraction.

Extreme cases of dedifferentiation at 72 hours are shown in Figs. 2, *d* and *e*. In Fig. 2, *d*, the general turgescence or tone, and the transparent appearance of the aboral region is preserved, but the oral region has rounded off, and all traces of the characteristic form of the oral half of the animal, including the ciliated band, have disappeared. The stomach too is affected, all parts of the gut being now reduced, with thick epithelium.

Finally in Fig. 2, *e*, we have a specimen with complete dedifferentiation of the oral region. The aboral region, however, is also markedly affected, and the whole body is filled with a nearly opaque mass of cells, within which only faint traces of organs are visible. The skeleton is reduced to two simple clubbed rods. In this condition the animal much resembles a dedifferentiated *Clavellina*, and the resemblance would externally be almost complete if it were not for the presence of the spicules, which prevent the retraction of the aboral region and the assumption of the spheroidal form.

At 96 hours, most of the specimens showed signs of partial death. This manifested itself in the oral region by a disintegration of some of the tissue, and the protrusion of the skeleton. The gut was in all cases contracted. The aboral region was usually contracted, but with no trace of disintegration. (Fig. 3, *a* and *b*.) Most of them were transferred to sea-water. Those that remained in the solution were all dead after 120 hours, with aboral as well as oral disintegration, but very little or no disintegration of the gut. Slight movement due to ciliary action, and occasional contractions of the œsophagus were seen up to 96 hours.

One interesting occurrence was noted after 72 hours. Two plutei had grown together, the left anal arm of one having completely fused with the aboral region of the other, the skeletons of the two individuals overlapping in the common region (Fig.

4). The pair was isolated in sea-water, but was dead by 120 hours.

The progress of dedifferentiation in $HgCl_2$ *n*/2,000,000 was similar but considerably slower.

This is shown in Fig. 5, which represents an advanced specimen after 120 hours (by which time all those in *n*/1,000,000 were dead). An abnormal addition to the skeleton is seen. This may perhaps be due to the deposition in the aboral half of calcium carbonate resorbed in the arms, or to the fact of the spicule-secreting tissue being less affected than the rest, as Child ('16) suggests. The mouth was, I think, closed; the œsophagus was swollen. Most individuals after 120 hours were in the same stage of dedifferentiation as shown by the *n*/1,000,000 culture after 48 hours.

Partial death was not seen till 144 hours; complete death had occurred by 168 hours. As in the *n*/1,000,000 culture, the still-living portion when partial death had set in was very narrow, the spicules being nearly parallel. This narrowness is a result of all regions being affected simultaneously.

The controls for this experiment remained very healthy and off the bottom for 96 hours. The culture was rather crowded and not fed. After 120 hours, most were on the bottom, and were showing a certain amount of arm-resorption (though less than that shown in *n*/1,000,000 after 24 hours). One was seen with 3 arms reduced to minute knobs, but the fourth perfect. This was never seen in the $HgCl_2$ cultures, although there the arms of one side might be resorbed faster than those of the other.

The resorption in the controls might have been due either to starvation, or to the accumulation of toxic products, or both, but tests were not made to determine the point. Runnström's results ('17), show that either alone can cause dedifferentiation.

All were on the bottom after 144 hours. They remained in this position with almost total absence of arms, but healthy in other respects, for 5–7 days longer.

F. Nine-day plutei from the main control culture (fed on *Nitzchia*) were transferred to $HgCl_2$ *n*/1,000,000.

The appearance of the more advanced of these is shown in Fig. 6. All had developed the third pair of arms.

After 24 hours all had become somewhat reduced in size, with slightly denser appearance. The gut too was shrunken. The arms, especially the third pair, usually showed slight reduction in size.

After 48 hours, marked changes had occurred (Fig. 7). The third pair of arms had in all cases totally disappeared, and the others showed considerable but variable resorption. The previously noted swelling of the trunk and aboral region was present to a greater or less degree. The most interesting change was seen in the gut, which was always contracted, and contained a greater or less number of small round bodies. On examination with a higher power, these proved to be cells, practically spherical, and not cohering. They had presumably migrated out of the stomach epithelium into the lumen.

This is paralleled by the migration of the cells out of the aboral ectoderm in Expt. *C* above (pp. 212–213), and by the behavior of the tissues in organisms that dedifferentiate by resorption, as in *Perophora* (Huxley, '21 *b*) and Hydroids. (Loeb, '00. Huxley and de Beer, in press.)

After 72 hours, dedifferentiation had progressed much further (Figs. 8, *a* and *b*). The size is much less, the arms very small and extremely dense, the gut quite packed with cells, and much contracted. The contraction of the gut has expelled some of the cells at the anus and sometimes also at the mouth. The trunk and aboral regions are sometimes swollen, more usually contracted. A fair number of plutei had died.

At this stage the survivors were replaced in sea-water.

KCN. Experiments on 4-day plutei in KCN *n*/100,000 and *n*/200,000 gave on the whole similar results to those in $HgCl_2$ *n*/1,000,000. Disintegration of the trunk ectoderm was never observed, and is possibly a specific effect of Hg (*cf.* Child, '17). Arm-resorption was not quite so rapid.

4. Experiments on Recovery.

These are very incomplete, owing to lack of material. Through the courtesy of Mr. J. Gray, who independently observed dedifferentiation phenomena, in larvæ treated with citrates, I am enabled to state that in this case some degree of recovery at least is possible. It would here appear that forms which have resorbed

their arms (similar to my Fig. 1, *b*, or 1, *d*) are able to regenerate them, while those which have dedifferentiated to a completely spheroidal mass can probably redifferentiate to a pre-pluteus (helmet-shaped) stage at least. Mr. Gray, however, proposes to investigate the matter further, and I merely quote his preliminary results in order to show that dedifferentiation does not preclude recovery, at any rate to some degree. As is well known, dedifferentiation in *Clavellina* and in *Protozoa* does not preclude complete recovery (Driesch, '06; Lund, '17, etc.).

The observations I have made, however, on forms dedifferentiated by KCN and $HgCl_2$ (in which case dedifferentiation appears to be slower than when caused by citrates) have not yet shown recovery, though many specimens lived for weeks in sea-water, and ingested food. From the present results, it would seem as if the poison had robbed the organism of its capacity for development, but not of its capacity for maintaining life. I must emphasize that owing to lack of more ripe *Echini*, I was unable to do more than carry out preliminary observations, and that systematic experiments on the subject are in view.

That being so, I will record my results in the briefest possible manner. Most moderately-dedifferentiated forms (*i.e.*, with ciliated band, but arms absent or vestigial) transferred to sea-water from KCN remained alive and in approximately the same condition for over 4 weeks. They kept on the bottom, and retained a slight motility. In one culture the beginnings of recovery were noted in the shape of increased motility and of a few larvæ beginning to swim freely once more, but after a few days they again reverted to their former condition. In all cultures a slight *progressive dedifferentiation* was noted after 1 to 2 weeks, and towards the end of the 4 weeks, a larger proportion of spheroidal forms was found.

On adding *Nitzchia* to two cultures, it was found that diatoms were ingested by many of the dedifferentiated larvæ. These cultures lived better than those without *Nitzchia*.

A number of these larvæ were also placed, after 2 weeks, in a jar with *Nitzchia* and a stirring apparatus; but 3 weeks later none could be discovered.

The history of those transferred to sea-water from $HgCl_2$ is

similar in essentials. The larvæ lived up to 3½ weeks. Some ingested *Nitzchia* when this was provided. Progressive dedifferentiation was, I think, more pronounced in these $HgCl_2$-treated larvæ.

Figures are given of some of the types seen during this progressive dedifferentiation. Fig. 11 shows a larva which has completely lost its arms and also its ciliated band, together with the antero-lateral and transverse skeleton on one side. The remainder of the skeleton permits it to retain some of its characteristic form. The gut and its epithelium are contracted, and its spatial relations altered. The body-cavity is clear, with a few clumps, some of pale cells, others of red pigment-cells. The general appearance is much clearer and less full of cell clumps than in larvæ in advanced stages of dedifferentiation still in the toxic solution.

Fig. 12 shows a further stage of loss of form. Here the outline is simply spheroidal. The broad œsophagus, contracted stomach and thin intestine lie approximately in a straight line. The body-cavity is very clear, with the exception of a few large clumps of cells. The aboral clubs have been broken off from the rest of the skeleton. This breaking of the skeleton, it should be noted, was frequently seen in forms where the aboral and trunk regions were much dilated. The change of form of the aboral region exerts a pressure inwards on the rods, at right angles to their axis, and snaps the ends off; at first they lie in the position in which they have been broken, thus making it easy to see how breakage occurred. Once this happens, the oral ends of the rods will no longer be pressed against the body wall, and the whole skeleton thus ceases to function as a support. This is clearly seen in Fig. 12, *a* and *b*.

The general appearance of the tissues in this larva, and indeed in most of those in the recovery experiments, is perfectly healthy. Even after the skeleton has disappeared and the spheroidal form has been assumed, traces of the "lip" or pre-oral lobe may often be seen near the mouth. This also, however, has disappeared in the larvæ shown in Fig. 12.

A very advanced stage of dedifferentiation is seen in Fig. 13 *c*. The larva had shrunk considerably in size. This is apparently due to the contraction of the ectoderm, which was cuboidal in-

stead of flattened. No trace of skeleton was present. (The animal was examined both *in vivo* and as a stained preparation.) Large, brownish aggregations were seen in the interior, together with an apparently closed and solid pale vesicle, perhaps the stomach. Curious irregularities of the ectoderm were observed at one pole. These were also seen in several other specimens (*cf.* Fig. 12 *b*).

The general resemblance of this specimen to much-dedifferentiated individuals of the Ascidian *Clavellina* is striking (Driesch, '06; Schultz, '07; and my own unpublished observations). There are numerous points of difference, as one would expect in such widely different organisms, but the following essential similarities exist: (1) the assumption of the spheroidal form; (2) the aggregation of free cells to form dark masses; (3) the regression of epithelial cells to the cuboidal condition; (4) the conversion of internal structures into closed vesicles; (5) the congested condition of the body spaces, consequent upon contraction.

5. On the General Effect of Mercury in Dilute Solutions.

In order to get some more accurate idea of the processes occurring in a weak solution of a mercury salt, some experiments were carried out on the gill of *Mytilus*. I have to thank Mr. J. Gray, of King's College, Cambridge, for some suggestions.

A preliminary test with various strengths of $HgCl_2$ solution showed that in very weak solutions marked disintegration of the tissue took place before ciliary action was stopped.

The point to be tested was whether the effect of Hg^+ ions was proportional to the *strength of solution* used, or was a progressive effect, proportional to the *total amount* of mercury in the solution.

(*A*) Five finger bowls were prepared, and 4 pieces of gill placed in each. One contained 50 c.c. of sea-water as control; the others 50 c.c. of $n/375{,}000$ $HgCl_2$. The solution in Nos. 1 and 2 was not changed. In No. 3, it was changed after 2, 4, 6, 8 hours and again after 24 hours. In No. 4, it was changed every ½ hour for 8 hours, and again after 24 hours.

After 3 hours, there was slightly more disintegration in No. 4 than elsewhere. After 6 hours, all the pieces in No. 4 had their

terminal rounded portion markedly disintegrated, and the current produced by the lateral cilia had ceased.

In Nos. 1 and 2, one piece in each was similar, but the others had scarcely begun to disintegrate. No. 3 was intermediate. In Nos. 1, 2, and 3 the current produced by the laterals was still evident.

After 24 hours, No. 4 was very markedly disintegrated, and no ciliary movement was visible. In Nos. 1 and 2, 4 of the 8 pieces showed ciliary movement, and the disintegration was not so marked as in No. 4. No. 3 was intermediate. The conclusion was apparently to be drawn that it is the total amount and not the concentration of the Hg^{+} ions present that is operative; but the concentration used was apparently too high. The experiment was therefore repeated in a modified form: (*B*) Five finger bowls were prepared with 5 pieces of gill in each. In them were placed respectively 5, 20, 50, 100 and 300 c.c. of a *n*/1,500,000 $HgCl_2$ solution.

The following table gives the results observed (+ = strong ciliary action; ⊕ = moderate; ⊕⊕ = faint; ⊕○ = very faint; ○ = no ciliary action. ((D)) = slight disintegration; (D) = medium; D = marked disintegration.

TABLE II.

No.	c.c.	Time in Hours.					
		3	21	27	45	70	
1.....	5	+	+	+	⊕	⊕	All proximal cut ends healed.
2.....	20	+	+	+	⊕	⊕ ((D))	
3.....	50	+	⊕ ((D))	⊕ (D)	⊕⊕ (D)	.....[1]	All proximal ends unhealed; dead after 45–70 hrs.
4.....	100	+	⊕ ((D))	⊕⊕ D	⊕⊕ D	.....[1]	
5.....	300	+	⊕ ((D))	⊕⊕ D	⊕⊕ D	⊕○ D	

It will be seen that the effect of the $HgCl_2$ was a function of the total amount used, all the solutions being of the same strength. The death of the proximal portions of the filaments was very striking in the large bulks of solution.

[1] Numbers 3 and 4 were not observed at 70 hours, owing to an oversight.

There was, however, very little difference between the effect of Nos. 4 and 5. That is to say, the maximum effect possible with a solution as weak as $n/1{,}500{,}000$ is, with the amount of tissue used in the experiment, attained with a total bulk of about 100 c.c.

In any experiments concerning the effects of salts of the heavy metals upon living cells, it will therefore be necessary in every case to consider not only the strength but also the amount of the solution, and further the amount of living tissue on which experiments are being carried out. Failing this, the results obtained will not be comparable. However, since in the experiments on plutei here recorded, the bulk of $HgCl_2$ solution was large in comparison to the bulk of the plutei, and since the results are only qualitative, they are not invalidated by the conclusions just reached.

6. Discussion.

A. *Maintenance of Form.*

Recent work is coming to show more and more clearly how organic form is the product of an equilibrium between constitution (internal forces) and environment. This applies equally well to whole organisms or to their parts. In order that the typical form may be maintained, a particular complex of environmental stimuli is necessary.

In the case of Ascidians such as *Clavellina* (Driesch, '06; Schultz, '07) and *Perophora* (Huxley, '21 *b*) whole zoöids below a certain size when exposed to unfavorable agencies, such as accumulated waste products or dilute solutions of KCN, are unable to maintain their form, and undergo what is known as dedifferentiation.

The same is true of Hydroid polyps (Loeb, '00). In Hydroid polyps we (Huxley & De Beer, in the press) have succeeded in finding a quantitative relation between the rapidity of the form-changes and the concentration of the toxic solutions employed; this is also seen in the present study (Table I.).

The encystment of *Protozoa* in times of drought or cold is an example of the same phenomenon.

The remarkable dedifferentiation of the hydriform larva of

Gonionemus observed by Perkins ('02) should again be mentioned here.

As regards parts of organisms, attention may be called to the phenomena seen in sponges (Minchin, '00, p. 29). When an Ascon type of sponge contracts, the internal environment is altered, and the collar cells are unable to maintain their typical form, losing their collar and flagellum and becoming spheroidal. See also Huxley ('21 *a*, p. 313).

The familiar resorption of many grafted tissues may also be mentioned. Muscle fibers, like *Clavellina*, also dedifferentiate preparatory to regeneration when cut across. (Towle ('01).)

Even the form of mental organization is subject to the same limitation. In certain "shell-shock" and other cases, strain and unfavorable environment render the higher part of the mental organization unable to maintain itself, resulting in what is known as *regression*. See Nichol ('20).

We may thus say that maintenance of normal form is possible only in certain environments. Certain stimuli result in what may be called hyper-typical form: *e.g.*, in regenerating Planarians, high temperature produces forms with exaggerated heads (Lillie and Knowlton, '97; Child, '15, p. 138). On the other hand, many unfavorable stimuli do not permit the establishment of the type at all: they result in infra-typical form—*e.g.*, cold in regenerating Planarians; below a certain temperature, no head is formed (*auctt: cit.*).

B. *Dedifferentiation and Metamorphosis.*

The resemblance of the phenomena here described to those occurring at the metamorphosis of the pluteus is very striking. According to MacBride ('02) the course of the process is as follows: The larva sinks to the bottom, presumably as a direct result of the weight of the growing *Echinus* rudiment. The arms are next resorbed, those on the same side as the *Echinus* rudiment first. The larval œsophagus contracts. The ectoderm of the ciliated epaulettes is "invaginated" and devoured by amœbocytes (the description, however, does not negative the possibility that the cells of the epaulettes may migrate out of the tissues, rather than amœbocytes migrate in). During arm-resorption, the ectoderm

of the arms shrinks, leaving the spines exposed "exactly as in unhealthy larvæ at all stages of development." The oral lobe and the outer part of the œsophagus disappear. The inner part of the œsophagus persists for a time as a completely closed tube. Very remarkable changes occur in the stomach. In the late larva it is highly turgid, with cells intermediate between cubical and flattened. It now loses its turgidity; the walls become very thick, and eventually folded, the lumen often almost disappearing. The cells are stated to multiply with great rapidity, but definite proof of this is not given, and quite possibly the appearance of increased number is due to the contraction of the wall; this point deserves re-investigation. The cells round themselves off, and many migrate into the surrounding jelly. The new stomach is reconstituted from the residue.

While this has been occurring, all resemblance to an Echinopluteus form has disappeared, and the animal becomes almost hemispherical.

The essential points to be noticed are as follows:

(1) The resorption of the arms. This appears to be identical with what we have seen in larvæ placed in toxic solutions.
(2) The dedifferentiation of the specialized larval organs, the epaulettes. These are not present in the earlier larvæ used by me.
(3) The loss of the general form of the larval part of the organism, and its approximation to the segment of a spheroid.
(4) The contraction of the œsophageal tissue.
(5) The closure of the mouth and formation of a closed vesicle from the remains of the œsophagus.
(6) The contraction (loss of turgidity) of the stomach.
(7) The migration of some of the cells of the stomach out of the tissues. This was paralleled in my experiments, though there the cells migrated inwards to the lumen, instead of outwards to the body-cavity.

As far as the destruction of larval organs goes, we can assert that the dedifferentiation caused by toxic solutions and the reduction at metamorphosis are closely and essentially similar. The difference between the end-results is presumably due to the fact

that at metamorphosis there is present a new organic system, the developing *Echinus* rudiment, which was absent in the subjects of the dedifferentiation experiments. We may suppose that the metamorphic changes in Echinoids are normally initiated somewhat as follows: the inherited constitution of the animal leads to the production of the *Echinus* rudiment. The weight of this leads directly to the sinking of the larva to the bottom. The subsequent changes would then be due to two causes: (1) The conditions at the bottom are directly unfavorable to the larval organs, which therefore are unable to maintain themselves, and so start dedifferentiating. (2) The developing adult organs are not inhibited by the benthic environment, and their continued growth and consequent demands for nutrition accelerate the dissolution of the larval system.

The stomach is an organ which is remodelled. It is unable to maintain itself in its typical larval form, and regular dedifferentiation-changes start in it. But the activities of the adult organs provide a new internal environment, and the remains of its tissues, entering into equilibrium with this, form the rudiment of the adult stomach.

It should follow from these considerations that precocious metamorphosis should be induced by placing larvæ with a developing *Echinus* rudiment in dilute toxic solutions. This conclusion it is intended to test by experiment. Meanwhile Professor MacBride informs me in conversation that those of his cultures in which conditions are not optimum, do as a matter of fact exhibit precocious metamorphosis. In such conditions, the larvæ sink to the bottom while the *Echinus* rudiment is still in a stage much less advanced than that which it possesses at metamorphosis in the best cultures. In spite of this, metamorphosis takes place, but leads to the production of small, weakly, under-developed Echini. I am grateful to Professor MacBride for informing me of this confirmation of my theoretical considerations.

Loeb ('00) made the suggestion that the retrogressive changes of histolysis in metamorphosis were comparable to dedifferentiation as seen in Hydroids, but I am not aware that the similarity between dedifferentiational and metamorphic changes in one and the same species has yet been pointed out, as here in *Echinus*.

If these considerations prove to hold good, it will follow that phagocytosis is a secondary phenomenon in the metamorphosis of Echinoids, and probably of other groups. It will, however, obviously be our next task to test by experiment the hypothesis that the dedifferentiation of larval tissues is the essential factor initiating Echinoid metamorphosis.

C. *Axial Gradients and Surface-effects.*

Child's theory of axial gradients and the differential susceptibility along them has been set out at length in his books ('15, etc.) and papers, and it is unnecessary to enter into it here.

I would like to point out, however, some facts which may lead to a modification of some minor parts of the theory.

Child measures and delimits his "metabolic gradients" by means of the differential susceptibility of different organs to toxic agencies. In certain cases this gives concordant and uniform results. In other cases the susceptibility of an organ is found to vary during development in a way which is not to be explained without demanding a very considerable elasticity from the theory.

The general bases of the theory appear to be founded on solid enough foundations, the main difficulty being that they are perhaps too general, the term "metabolic rate," for instance, being only capable of application in an unanalysed sense, as an expression of general total activity. But numerous exceptions, such as the unpredictable variation in susceptibility of organs above alluded to, can I think be explained by reference to another and simpler notion. That is, that cells are more susceptible and more prone to dedifferentiation in proportion to the amount of surface which they are exposing. We can put this in another way, and say that a cell maintains its form with greater difficulty when its surface is large than when it is small.

This statement is based on numerous facts, including the following observed by myself, as well as being deducible from theoretical considerations.

(1) In the Ascidian *Clavellina,* the parts first showing dedifferentiation, and finally most dedifferentiated, are those where the normal cells expose a great deal of surface (pharynx and atrium).

(2) The same is true of the related form *Perophora,* in spite of its different mode of dedifferentiation.

(3) In *Perophora,* the stolon-ectoderm is usually flat. When treated with toxic agents of a certain strength, it becomes cuboidal.

(4) In sponges, the collar-cells exposed to very mildly toxic agents retract their collars. Further toxicity causes the assumption of the spheroidal form by the cell. (Huxley, '21*a*.)

(5) In dissociation experiments, the shock of mechanical separation causes the assumption of the spheroidal form, and the loss of any differentiated structures such as collars, flagella, or pseudopodia, in all types of cell in sponges. (This is true also in other sponges (H. V. Wilson, '07) and in Cœlenterates (H. V. Wilson, '11; De Morgan and Drew, '14).)

(6) In the plutei here described, the gut is at first extremely resistant to toxic agents. Later, however, it becomes very susceptible to them. This alteration in susceptibility is accompanied by an alteration in appearance, the gut passing from a thick-walled organ to an extremely turgid, thin-walled one. This turgidity has also been noted by MacBride ('02); the cells in this latter condition are very much flattened, with a relatively enormous surface. One of the first effects of $HgCl_2$ solutions in later larvæ is to cause a shrinkage of the gut and a separation and rounding-up of some of its cells.

It is of course presumable that high energy-consumption is necessary to maintain a cell in a flattened condition, or in any other involving a large surface area—as would indeed be expected from the laws of surface-tension. But to say that a high "metabolic rate" thus produced is to be reckoned in the same category as the high metabolic rate of a "dominant" region as defined by Child, is to reduce the value of the whole very important conception of physiological dominance.

Further, in cells grown in vitro, Holmes ('14) notes as a general rule that any unfavorable condition leads to the abandonment of an extended for a spheroidal shape. Numerous other instances could be cited, but the phenomenon is so widespread as to be familiar to all.

We may therefore say that, apart altogether from the question

of axial gradients, the susceptibility of cells to toxic agents, and their readiness to dedifferentiate, are in part functions of their surface area.

D. *Previous References to Dedifferentiation in Echinoid Larvæ.*

Vernon ('94) found that various environmental factors had a marked effect on the arm-length of plutei (the experiments were continued up to the 8-day stage). The effect, whether caused by actual resorption of tissue present, or failure to grow beyond a certain length, means that equilibrium with these slightly unfavorable conditions could only be maintained by arms of a shorter length than normal.

Robertson ('13) found that *Strongylocentrotus* blastulæ transferred to a medium containing 0.15 per cent. lecithin for 24 hours and then returned to sea-water, not only were very much retarded in development, but after becoming gastrulæ nearly lost their gut again before dying (figures and details are not given). He ascribes this result to a specific action of lecithin on growth-processes. From what we know of Echinoderm development, the result, in the absence of further data, might at least equally well be non-specific and due to dedifferentiation.

In addition, the literature abounds with notices that "unhealthy" larvæ may show shortened arms, sometimes with a terminal protrusion of spicules. (And see postscript, p. 230.)

E. *Recovery.*

The meager results on recovery merit one or two words. Future experiment must decide between one or two possibilities. Either the Hg^{+} ion damages only a fraction of the cells' protoplasm, which, if it does not exceed a certain critical value, may be repaired, and normal differentiation resumed; or repair is not possible, and the animal cannot differentiate further. The theoretical bearings of this second possibility have been discussed by Child ('16) and I do not intend to go into them at present. What is interesting in either eventuality is the extremely long period for which the dedifferentiated organisms can remain not merely

alive, but, it should be emphasized, with apparently quite healthy tissues.

The fact that such larvæ ingested diatoms in a normal fashion is noteworthy. The possibility is thus raised of producing, by environmental changes, permanent larval forms. (*Cf.* Perkins, '02.) Such permanent larval forms of small size, but hereditarily determined, are of course known in many species, especially in the male sex.

The action of mercury is apparently to precipitate and put out of action a definite quantity of the living molecules in the cell.

Postscript.

Owing to delay in the arrival of German periodicals after the war, I did not see the important paper by Runnström ('17) until I had not only completed the work here recorded, but also written the paper. Runnström has demonstrated that young Echinoid plutei treated with $ZnSo_4$ in very weak solutions undergo dedifferentiation-changes very similar to those observed by me. He also finds that dedifferentiation occurs spontaneously in a certain proportion of larvæ in every culture, especially from over-ripe eggs. The specimens noted by me on p. 213 above are doubtless of this category. Finally, he describes very similar dedifferentiation as the result of starvation, from which recovery is possible. Recovery is also possible in the $ZnSo_4$ larvæ when replaced in sea-water.

I will not summarize his results further, except to say that they show conclusively that dedifferentiation may be produced by many agencies, and may be reversible.

There are one or two points of general theoretical interest which may be noted. While remarking on the resemblance of the changes seen at metamorphosis to those produced by starvation, Runnström says that an *essential* difference between the two is the rapidity of the metamorphic changes. On the other hand, reduction by means of toxic agencies is more rapid, and it should be remembered that at metamorphosis the existence of rapidly growing imaginal organs will drain the rest of the organism very quickly. The rapidity of dedifferentiation in the zoöid of *Perophora* is very great—*once it has started*. The observations of Professor MacBride (p. 226) also corroborate strongly the idea

that toxic agencies are important in the initiation of metamorphosis. Cytolysing agencies are not necessary to explain resorptive dedifferentiation such as occurs in Hydroids and *Perophora;* they should therefore not be postulated, as is done tentatively by Runnström, to explain metamorphic changes in Echinoderms until definite proof can be adduced of their existence.

A further corroboration of the idea of metamorphosis as an upset of balance is to be seen in Runnström's own remarkable observations upon the formation of pedicellariæ. In much-reduced larvæ, more or less well-differentiated rudiments of pedicellariæ often appear *ab initio*. Once pedicellariæ have been formed, however, they are among the first organs to be dedifferentiated. This may be interpreted by supposing that the potency of producing pedicellariæ is present in larvæ, but normally inhibited until a certain stage by the demands of the existing organs. When these are reduced, however, the check is removed and the pedicellaria develops. Once developed, on the other hand, its cells are not actively growing, and are easily affected by unfavorable agencies. This is very similar, it would seem, to the behavior of larval and adult organs at metamorphosis.

With Runnström's general discussion I find myself in full agreement in all essentials, except that I do not consider that he has laid sufficient stress upon the direct inhibiting effect of poisons upon growing tissues, an effect which leads eventually to dedifferentiation by a different route from that consequent upon hunger.

April 6, 1921.

Summary.

1. The retardation of early *Echinus* development caused by toxic agents (KCN and $HgCl_2$) is noted.

2. When recovery took place on transference to sea-water after 48 hours in the toxic solutions (*i.e.*, in the blastula stage), plutei were formed. These were mostly very wide-angled; this was due to the greater power of recovery of the oral region.

3. On transference to sea-water after 72 hours' treatment with the poisons (*i.e.*, in the gastrula stage), development only took place as far as the pre-pluteus stage. Considerable power of re-

covery is thus lost by prolonging the treatment with poisons from 48 to 72 hours.

4. Gastrulæ killed by weak toxic solutions show *differential death,* the enteron being more resistant than the rest of the tissues.

5. In very weak solutions, progressive *dedifferentiation of plutei* occurs. The arms are first resorbed, then the ciliated band and oral lobe disappear, then the gut contracts. In early stages, the trunk and aboral end are dilated, in later stages they are contracted and the whole body is filled with cells. The process is similar in all essentials to that seen in the dedifferentiation of the Ascidian *Clavellina.*

6. It could not be decided whether full *recovery* is possible for plutei thus treated. Armless forms replaced in sea-water remained alive and motile for 3½ to 4 weeks, and ingested diatoms. Some of them showed *further dedifferentiation* in the sea-water, finally reaching a spheroidal state, with spicules extremely reduced or absent, and gut reduced to a closed vesicle or vesicles.

7. The process of dedifferentiation appears to be essentially identical with that initiating *metamorphosis* and resulting in the destruction of the larval organs in Echinoids. This is probably true also for metamorphosis in other groups.

8. Echinoid metamorphosis, and possibly other types of metamorphosis also, would therefore appear to be *initiated* by dedifferentiation-changes in the larval organs, not by autolysis or phagocytosis, which are secondary phenomena.

9. Ceteris paribus, tissues with *large cell-surface* are more susceptible to unfavorable influences and more prone to dedifferentiation than are those with a small area of surface per cell.

10. The effect of mercury salts in dilute solutions is shown to depend upon the *total amount* present as well as upon the *concentration.*

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LIST OF FIGURES.

All figures were drawn from life with the aid of an Abbé camera lucida, at table level. All are drawn with the combination of a Reichert 1/3″ lens and a No. 4 ocular, with the exception of Fig. 2, *b* (1/6″ lens + 4 ocular), and Fig. 10 (⅙″ lens + 2 ocular, reduced to half-size). Figs. 6 and 10 have been reduced to one-half diameter, all others to two-thirds.

Plate I.

Figs. 1–4. 5-day plutei in $HgCl_2$, *n*/1,000,000.

Fig. 1. After 24 hours.

Fig. 1*a*. The most frequent type, with arms considerably resorbed, aboral end dilated. Anal view.

Fig. 1*b*. Similar, but arms more resorbed. The red pigment grains are here represented. Anal view.

Fig. 1*c*. Similar, but not quite so much arm-resorption; marked dilatation of the trunk-region. The epithelium of the trunk and aboral regions is exceptionally thin. Lateral view.

Fig. 1*d*. More complete dedifferentiation. The trunk and aboral regions are contracted instead of dilated; consequently the aboral ends of the spicules are not separated, and the trunk epithelium is thicker than normal. Anal view.

Fig. 1*e*. Similar, but with more pronounced arm-resorption. Anterior view.

Fig 2. After 72 hours.

Fig. 2*a*. Specimen with arms vestigial and dense, thin form, and clumping of mesenchyme. The stomach is normal, the intestine and œsophagus shrunken. Lateral view.

Fig. 2*b*. Aboral end of the same, under higher magnification. The light masses are composed of pale yellow cells with few granules. The dark bodies are red pigment. Quite aborally is a mass of slightly different clear cells.

Fig. 2*c*. The commonest type. The anal arms are totally absent, the oral arms and lobe almost entirely so. The skeleton of the oral arms is just protruding. The trunk and stomach are swollen, but the intestine contracted. Oblique anal view.

Fig. 2*d*. No trace of arms or of the form-differentiation of the oral end remains. On one side, the skeleton of the oral and anal arms has disappeared. The gut is shrunken, and its parts lie nearly in a straight line. Oblique anal view.

Fig. 2*e*. Complete dedifferentiation of the form of both oral and trunk regions. Body opaque and dense, filled with clumped mesenchyme. Skeleton reduced to two straight rods. Lateral view.

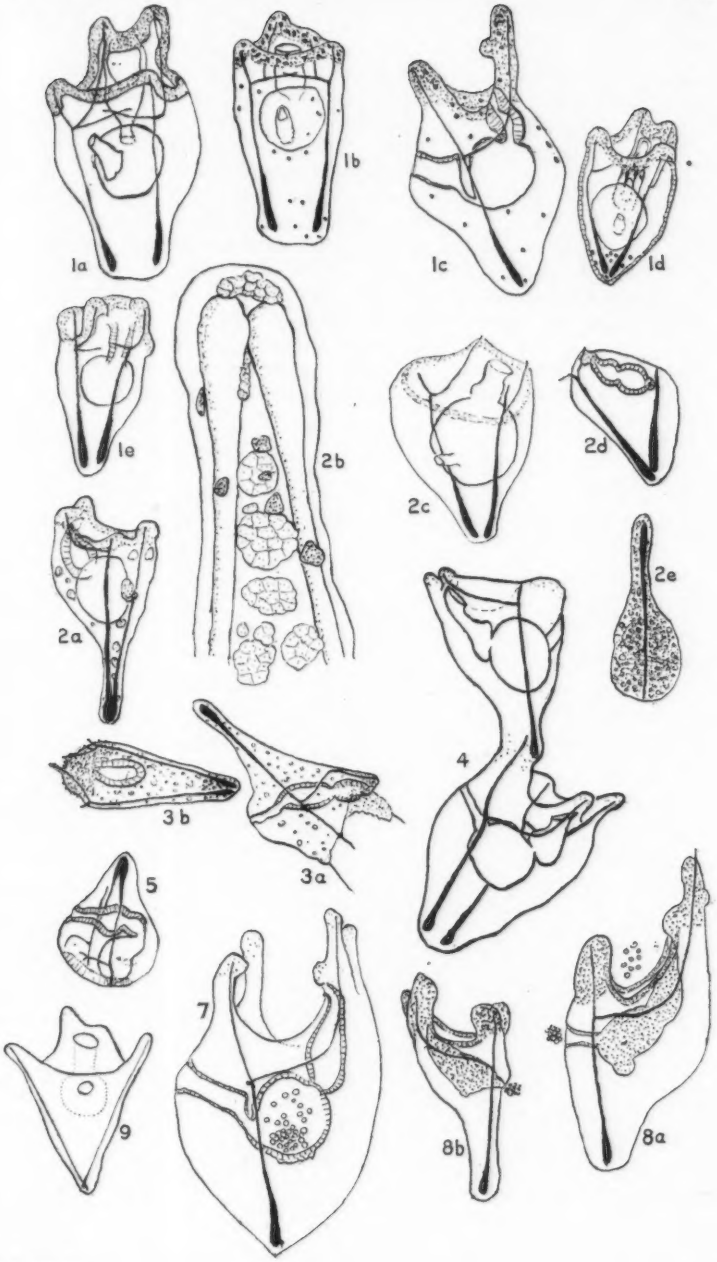
Fig. 3. After 96 hours.

Fig. 3*a*. Protrusion of skeleton, disintegration of oral arms only. Trunk region still somewhat expanded. Lateral view.

Fig. 3*b*. Specimen dedifferentiated in all regions, with incipient death-changes. A mass of red pigment aborally. Disintegration of whole oral region beginning. Anterior view.

Fig. 4. A pair of plutei found grown together after 72 hours. Lateral view.

Fig. 5. A 4-day pluteus in $HgCl_2$ *n*/2,000,000, after 120 hours. Note the remains of the ciliated band, the reduced stomach but dilated œsophagus, and the abnormal skeleton. Lateral view.



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FIGS. 6–8, AND 14. 9-day plutei in $HgCl_2$ *n*/1,000,000.

FIG. 7. After 48 hours, in *n*/1,000,000 $HgCl_2$. The third pair of arms has disappeared, the other arms markedly reduced; note the clubbing of one of the anal arms. The stomach contatins numerous cells migrated out of its walls. Aboral and trunk regions are swollen. Lateral view.

FIG. 8. After 72 hours in *n*/1,000,000 $HgCl_2$.

FIG. 8*a*. The arms are markedly dense, and very small. The skeleton of the oral arms protrude slightly. The remains of the oral lobe are visible. Stomach and œsophagus are quite dense. Cells have been extruded from both anus and mouth. The trunk is slightly swollen. Lateral view.

FIG. 8*b*. More advanced stage. The oral lobe has disappeared, the arms are dense and shorter, the trunk and aboral regions are affected. Cells have been extruded at the anus. Lateral view.

FIG. 9. Wide-angled pluteus. 2 days from fertilization in KCN *n*/100,000. 2 days in sea-water. Only the anal skeleton is drawn. Anal view.

Plate II.

Fig. 6. Outline of 9-day control pluteus when transferred to the solution *n*/1,000,000 $HgCl_2$. The skeleton is omitted. Anterior view.

Fig. 10. Differential disintegration of gastrula in *n*/1,000,000 $HgCl_2$. The nature of the spherical non-disintegrated mass was not ascertained.

Figs. 11–15. Stages of progressive dedifferentiation of plutei after replacement in sea-water from toxic solutions.

Fig. 11. Form without trace of ciliated band. Gut epithelium cuboidal (occasionally contracting). Trunk ectoderm thin. 2 days in KCN *n*/100,000, 17 days in sea-water. *m* = mouth.

Fig. 12. Completely spheroidal forms.

Fig. 12*a*. With aboral ends of spicules broken. Gut straight, with cuboidal epithelium. No trace of ciliated band, oral lobe, or typical form. Some branched mesenchyme cells. *m* = mouth. 2 days in $HgCl_2$ *n*/1,000,000, 10 days in sea-water.

Fig. 12*b*. Another specimen from the same vessel, 3 days later. Faintly motile. Gut reduced to a closed vesicle with cells aggregated round it. A curious projection with sharp lobes in oral region.

Fig. 13. Extreme reduction.

Fig. 13*a*. Non-motile, with remains of aboral ends of spicules, a dense mass of cells surrounding a gut-vesicle, and very thin ectoderm. 1 day in KCN *n*/25,000, 13 days in sea-water.

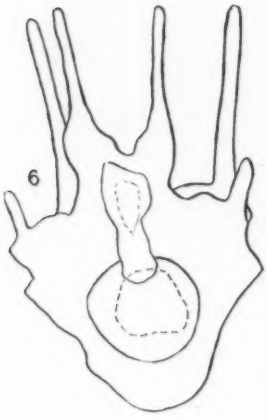
Fig. 13*b*. Still further reduction. Remains of only one spicule. Dense interior, with no visible trace of internal organs. 4 days in KCN *n*/25,000, 10 days in sea-water (with *Nitzchia*).

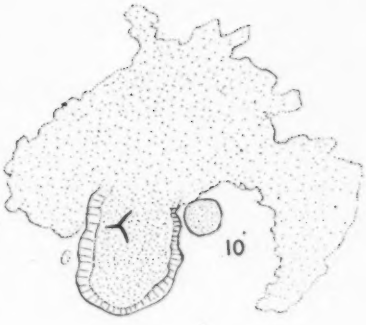
Fig. 13*c*. Faintly motile. No trace of spicules. Oral projection as in 12*b*. Several brown aggregations of cells, and a vesicle presumably derived from the gut. 2 days in $HgCl_2$ *n*/1,000,000, 30 days in sea-water (with *Nitzchia*).

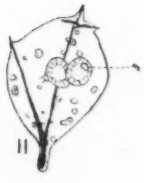
Fig. 14. A larva not quite so much dedifferentiated as 12*a*.

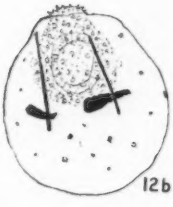
Fig. 15. A much-reduced larva, showing protrusion of the left spicule at both ends. Where it protrudes aborally, a ring of thickened ectoderm surrounds it. The other spicule is absent; presumably it has simply fallen out. The œsophagus has partially disintegrated. 3 days in $HgCl_2$, *n*/1,000,000, 2 days in sea-water.

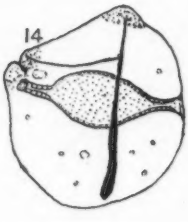
Fig. 16. A larva treated like that shown in Fig. 7.

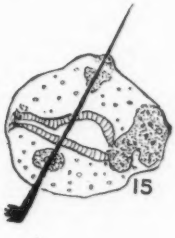


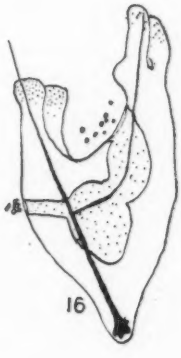
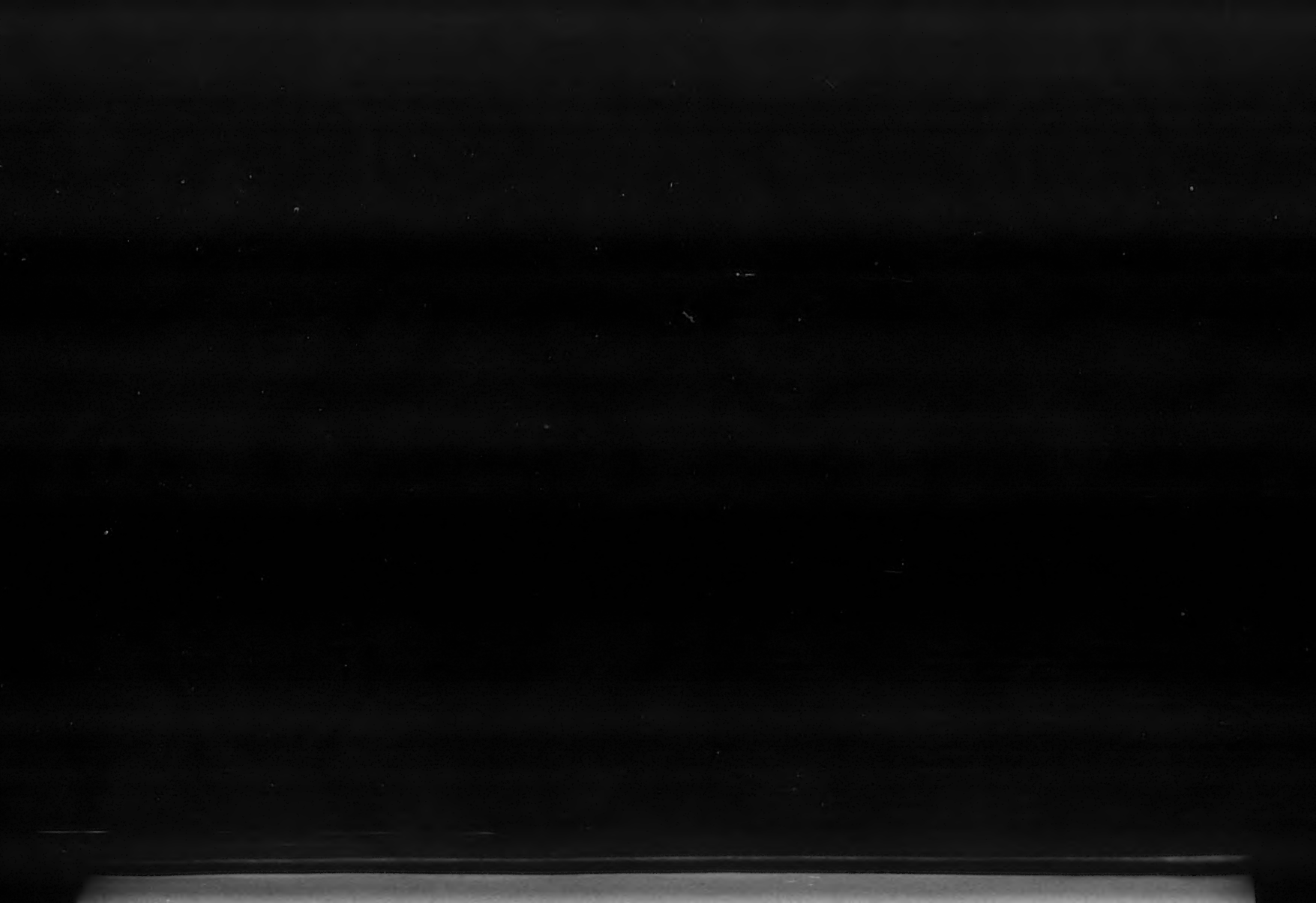








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